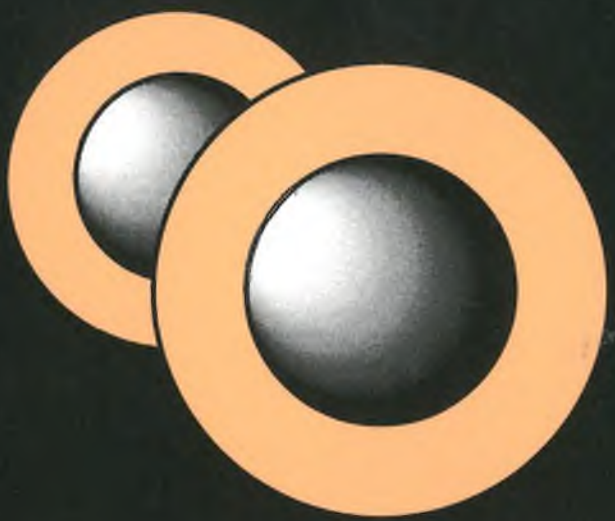
Mono-functionalised Cyclodextrins as Building Blocks for Supramolecular Systems



Fokke Venema

Mono-functionalised Cyclodextrins as Building Blocks for Supramolecular Systems

een wetenschappelijke proeve op het gebied van de
Natuurwetenschappen

Proefschrift

ter verkrijging van de graad van doctor aan de Katholieke Universiteit Nijmegen,
volgens besluit van het College van Decanen in het openbaar te verdedigen op
woensdag 17 januari 1996, des namiddags te 3.30 uur precies

door

Fokke Venema

Geboren op 26 februari 1966
te Harderwijk

Promotor	Prof. Dr. R.J.M. Nolte
Co-promotor	Dr. M.C. Feiters

The research described in this thesis was carried out with financial support of the Netherlands Technology Foundation (STW), Technical Science Branch of the Netherlands Organisation for Advanced and Pure Research (NWO)

ISBN 90-9008865-2

voor Opa

Dankwoord

Bij het afsluiten van vier jaar promotie-onderzoek door middel van het schrijven van een proefschrift besef je pas hoeveel mensen er direct of indirect by betrokken zijn geweest In dit dankwoord wil ik dan ook graag deze mensen met name noemen
Allereerst natuurlijk mijn promotor prof R J M Nolte Beste Roeland, het werken bij jou in de groep heb ik als zeer leerzaam en fascinerend ervaren De vrijheid die ik van je kreeg wat betreft de invulling van mijn onderzoek heb ik zeer gewaardeerd Jouw stimulerend enthousiasme heeft ertoe bijgedragen dat ik de afgelopen vier jaar met veel plezier bezig ben geweest Mijn copromotor, dr Martin Feiters, wil ik graag bedanken voor zijn kritische geluiden als mijn enthousiasme afgeremd moest worden en voor de oorstrelende geluiden met de titels "bonkende buren" en "romantische pianoklanken voor bij de centrale verwarming"

Veel van het werk dat in dit proefschrift is beschreven werd verricht door vier hoofdvak-studenten die bij mij hebben willen werken Ik denk met veel plezier terug aan de diverse leden van de "Cyclodextrin Task Force" Chantal Baselier, jouw werk aan dimeren blijkt de solide basis voor dit proefschrift te hebben gevormd Het is dan ook goed om te weten dat de volgende generatie (scheikunde-?)studenten alvast door jou wordt voorbewerkt René Uittenbogaard, jouw voorliefde voor raadsels werd als het ware door de moleculen die jij synthetiseerde aangevoeld Weinig van jouw verbindingen gedroegen zich zoals dat hoorde, maar met de raadsels waarmee deze moleculen ons opzadelden, bleken we later ons voordeel te kunnen doen Bart Nelissen en Menno de Jong, toen jullie begonnen moesten er nog de nodige hoofdstukken van dit proefschrift worden gevuld Jullie beloofden braaf daar een slordige drie van voor jullie rekening te nemen Ik weet niet of het de stimulerende werking van de vaak onbereikbare flessen is geweest, maar jullie werk aan lichtjes en luchtjes, respectievelijk, heeft voor de nodige vulling van dit boekje gezorgd

Mijn werkzaamheden in het UL hebben zich afgespeeld op drie verschillende verdiepingen, waardoor ik heb kunnen profiteren van de gezelligheid en collegialiteit van een hele serie studenten en collega's Al deze mensen hebben ertoe bijgedragen dat ik iedere dag met plezier aan de slag ging Daarvoor ben ik jullie erg dankbaar!

Het onderzoek dat ik heb uitgevoerd was onderdeel van een samenwerkingsverband tussen onze vakgroep en de vakgroep organische chemie van de Technische Universiteit van Twente Ik wil dan ook voor de prettige samenwerking bedanken prof David Reinhoudt, dr Johan Engbersen, dr Erik van Dienst, Irma von Piekartz-Eissink en Bianca Snellink-Ruel Ook de leden van de STW-gebruikers-commissie wil ik hierbij bedanken voor de interesse die zij hebben getoond in mijn werk
De technische, analytische en logistieke ondersteuning, die onmisbaar is wanneer je onderzoek probeert te doen, werd in mijn geval perfect verzorgd door de volgende mensen Heleen Amatdjais, Gerry Ariaans, Chris Kroon, Peter van Galen, Wim van Luyn, Pieter van der Meer en Sandra Tijdink Ook René Aben, die behalve tips en hulp bij van alles en nog wat, gratis levenswijsheden gaf, wil ik hierbij bedanken Deze taak (inclusief levens-wijsheden) werd na de verhuizing naar de eerste verdieping, perfect overgenomen door Hans

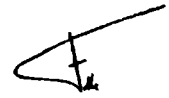
Adams, waardoor ik me meteen thuis voelde op de nieuwe zaal Ad Swolfs, iedere keer als je nog maar één meting voor me hoefde te doen, bleken er altijd weer minstens drie nieuwe experimenten uit voort te komen Bedankt voor de vele spectra die de geheime wereld van mijn moleculen een beetje ontsluierde

Dr Gordon Chittenden wil ik hierbij bedanken voor het verstrekken van suikeradviezen en de bijbehorende literatuur, dr René van Nostrum voor het corrigeren van de ruwe versie van dit proefschrift en Patricia Gosling voor het corrigeren van het Engels Also, I would like to thank Dr Alan Rowan for his contagious enthusiasm and for his contributions to Chapter 4 which also include the fancy computer images that coloured my lectures Ook wil ik Albert Schenning bedanken voor de "very stimulating discussions" waarvan gedeeltes terug zijn te vinden in een aantal hoofdstukken

De diversiteit van de hoofdstukken heeft ertoe geleid dat ik af en toe expertise "buiten de deur" heb moeten zoeken Hierdoor kreeg ik vaak een heel andere kijk op mijn verbindingen waarvoor ik de volgende mensen met name wil bedanken Prof Lutgarde Buydens, Dieneke Kleene (analytische chemie, Nijmegen) en dr Kees Koopal (TNO-voeding, Zeist) voor hun bijdragen aan het "beregeur-project" waarmee de basis werd gelegd voor hoofdstuk 6 Het cyclodextrine-polymeer uit ditzelfde hoofdstuk werd gekarakteriseerd m b v GPC-metingen door Dr K Brunt en E Slim van het NIKO-TNO in Groningen, waarvoor mijn dank Xavier Lautenslager wil ik bedanken voor het verrichten van de SPC-metingen aan de dansylverbindingen waarvan de resultaten zijn opgenomen in hoofdstuk 5 Also I would like to thank Dr Patrick Berthault and Dr Nicolaos Birlirakis from the Centre d'Etudes de Saclay, Gif sur Yvette, for their major NMR-contributions which are included in Chapter 2 Particularly the full assignment of nearly all proton signals, present in a cyclodextrin dimer, has really given us more insight in the process of self-inclusion of the spacer

Het leven buiten de chemie werd vooral aangenaam door de vele gezellige etentjes en vakanties met de "Utrechtse" vriendenclub Jullie hebben er voor gezorgd dat ik, naast mijn werk, ook de nodige ontspanning had Ook mijn zeil(kamp)-maatjes wil ik hier bedanken voor de vele aangename en vaak vermoeiende uren op en naast het water

Mijn vader en moeder wil ik bedanken voor de steun en de vrijheid die ze me hebben gegeven om te doen en laten waar ik zin in had Jullie interesse en die van mijn broers en zussen, inclusief aanhang, in mijn gefreubel waren bijzonder stimulerend Ook mijn opa wil ik hier bedanken voor de manier waarop hij mijn onderzoek heeft gevolgd en zelfs heeft aangevuld met extra artikelen
Als laatste wil ik natuurlijk Martine bedanken Jij hebt er op fantastische wijze voor gezorgd dat ik in de weekenden niet de hele tijd aan het boekje kon werken, waarvoor ik je erg dankbaar ben De ideale promotieomstandigheden die jij de afgelopen jaren hebt gecreeerd zal ik over een paar jaar proberen te evenaren.



Contents

Chapter 3
Synthesis of Novel Cyclodextrin Dimers and their Application in the Catalytic Epoxidation of Nerol

Chapter 4
Binding Studies of Cyclodextrin Dimers

Chapter 5
Dansyl-appended Cyclodextrins as Sensor Devices

Chapter 6
Detection of Organic Vapours in Air Using Cyclodextrin Coated Piezoelectric Crystals

CHAPTER 1

General introduction

1.1 Introduction

A relatively new topic in organic chemistry which is rapidly drawing more and more attention, deals with the complexation of organic molecules (guest molecules) inside other molecules (host molecules) which contain cavities, niches or pockets Such molecular complexations are the basis of the highly specific processes that occur in biology, such as the binding of a substrate to an enzyme or the recognition between DNA fragments To mimic and study these molecular recognition processes many researchers have investigated the interactions between synthetic receptor molecules and substrates This field of research has become widely known as host-guest chemistry [1] Lehn introduced the more general term supramolecular chemistry which was defined as the study of the structures and functions of complexes or aggregates that result from non-covalent interactions between molecules [2] The field of supramolecular chemistry includes many research areas, such as molecular recognition, self-assembly of molecules, (co-) catalysis by host-guest complexes and transport of electrons, protons and metal-ions [3] Several review articles dealing with a large variety of host molecules and their interactions with guest molecules have been published [4 5 6] In this chapter we will focus on cyclodextrins (CDs) and their inclusion complexes because these molecules are the subject of this thesis

Cyclodextrins were the first compounds to be studied with regard to their complexation behaviour and catalytic properties, the objective being to use them as enzyme mimics They are chiral and available as a homologous series of water soluble compounds, containing well-defined hydrophobic cavities Their chemical stability and low price have stimulated the search for new applications This thesis deals with the synthesis of new cyclodextrin derivatives which we have used in supramolecular systems to achieve (site-specific) complexation and catalysis In addition, we have applied these new compounds to develop a device for the detection of organic compounds in air and in solution

In this chapter a survey of the history of cyclodextrins will be given, followed by a description of the structure and physical properties of these molecules, their complexation behaviour and

the procedures to selectively modify cyclodextrins. Finally, the applications of CDs in supramolecular systems will be briefly reviewed.

1.2 History

The first publication on cyclodextrins appeared in 1891 by Villiers [7] who reported the isolation of two different crystalline compounds from the degradation of starch that had been treated with the bacterium *Bacillus amylobacter*. Villiers characterised the compounds by measuring their physical properties and called them "cellulosines". Several years later Schardinger published more physical data of these compounds.[8] He also succeeded in isolating the bacterium responsible for the formation of cyclodextrins (*Bacillus macerans*) which is to this day the most frequently used source of the enzyme by which CDs are produced. Due to his pioneering work CDs are sometimes referred to as Schardinger dextrins. Other names, which have been used in older literature, are cycloglucans, cyclomaltooligosaccharides and cyclo-amyloses. The cyclic structure of CDs was not recognised until 1938 when Freudenberg reported that cyclodextrins are built up from α-1,4-linked D-glucose units.[9] The most important property of cyclodextrins is their ability to form complexes with a variety of organic compounds. Schardinger already used this property in 1911 to precipitate his dextrins with alcohol and ether.[10] Only thirty years later, Freudenberg realised that these precipitates were the result of the formation of inclusion complexes.[11] Freudenberg's paper has led to an increasing number of articles describing the complexation properties of cyclodextrins. Also new CD derivatives were synthesised and the first article describing CDs as enzyme models was published by Cramer in 1953.[12] In the last twenty years the number of articles and patents dealing with CDs has steadily increased, as can be seen from Figure 1.1. In the following sections the structure and the properties of cyclodextrins will be described in more detail.

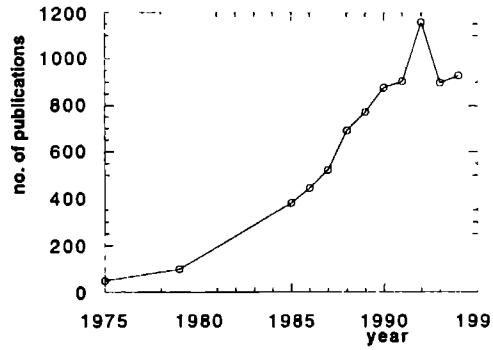


Figure 1.1 *Number of articles and patents on cyclodextrins per year (see refs 13-15).*

1.3 Structure and physical properties of cyclodextrins

Cyclodextrins are obtained by the degradation of starch through the action of the enzyme glycosyl transferase from *Bacillus macerans*.[16] This degradation yields a mixture of cyclodextrins which differ in size. In Figure 1.2 several representations of cyclodextrins are shown. As can be seen from this figure, CDs are cyclic oligosaccharides which are built up from D-glucopyranose units that are α-(1->4)-linked to each other. CDs are designated by a Greek letter to indicate the number of glucopyranose units in the ring: α-CD for six, β-CD for seven, γ-CD for eight units and so on.

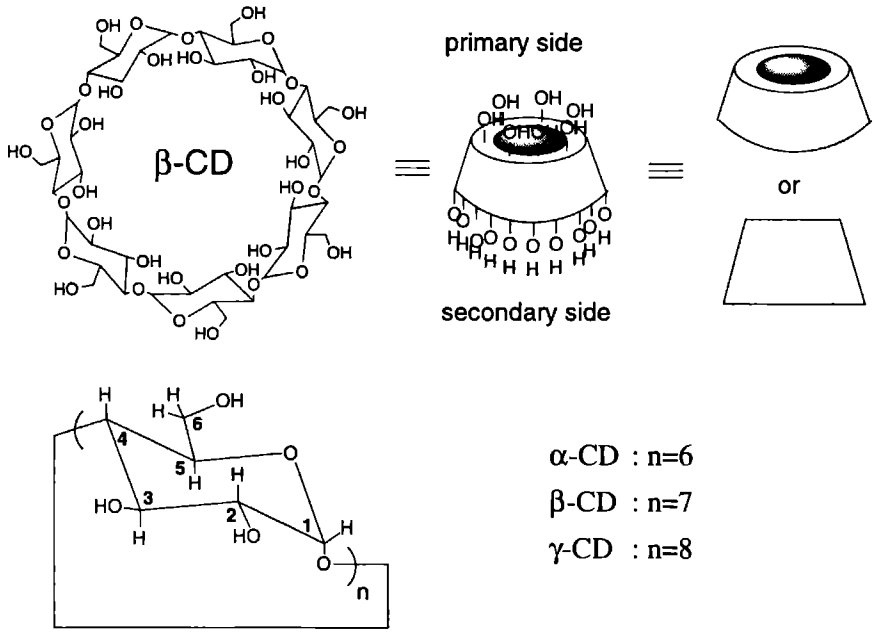


Figure 1.2 *The structure of cyclodextrins.*

The relative amount of the CDs formed in the degradation reaction of starch decreases in the order β-CD > α-CD > γ-CD. Very recently the enzyme responsible for the degradation of starch and the cyclisation reaction was modified by site-directed mutagenesis in such a way that a tyrosine unit in the active site was replaced by a leucine unit.[17] This modification caused a drastic change in the product composition: the amount of α-CD became negligible whereas that of γ-CD increased enormously. Because of the use of modified enzymes the price of γ-CD has become lower in the last five years.

δ-Cyclodextrin was described by French in 1961 [18] and its crystal structure was recently solved. It showed that δ-CD has an elliptic shape and not a ring structure.[19] Although larger

cyclodextrins, e g ε-CD, ζ-CD, η-CD and θ-CD, have been reported in the literature [20] these compounds could not be obtained in pure form but as mixtures containing CDs with branched cyclic structures One of these compounds, η-CD, was prepared very recently through the action of β-amylase, pullulanase, and yeast on solid CD Several purification steps yielded η-CD in pure form which was characterised by NMR and FAB-MS [21] It has been suggested that CDs smaller than α-CD cannot be formed for steric reasons [22] Watanabe et al , however, showed that it is possible to obtain a cyclomaltopentaose containing five glucopyranose units via a synthetic pathway involving many glycosidation reactions, although the overall yield was very low [23] A Greek prefix cannot be used to name this compound Lichtenthaler, therefore, suggested a new nomenclature for CDs allowing this derivative to be called *cyclo*-α(1->4)-glucopyranosyl-pentaoside [24] Although this nomenclature is very useful for the description of cyclodextrin derivatives (e g α-(1->6)-linked-analogues [25 26]) we will use the conventional Greek prefixes in this thesis

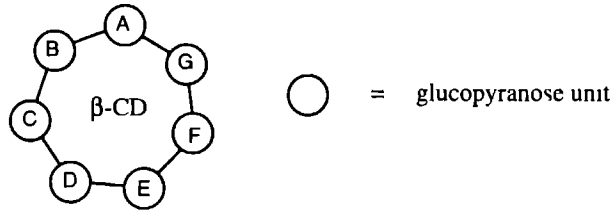


Figure 1.3 *Numbering of the glucopyranose units in β-CD, seen from the primary side*

If a cyclodextrin molecule contains more than one functional group, nomenclature rules are required to indicate the exact place of the substituents The CD rings (seen from the primary side), therefore, are numbered counterclockwise with A, B, C, etc , see Figure 1 3

Cyclodextrins have the shape of a bucket without a bottom and contain a cavity which is "V - shaped as can be seen from the schematic drawing in Figure 1 2 All glucose moieties are in the chair conformation The complete rotation of one glucose unit around the glycosidic bonds is restricted due to steric hindrance Because of this, the protons at C-3 and C-5 of the glucose units are always located in the CD cavity whereas those at C 1, C-2 and C-4 are pointing outwards The primary hydroxyl groups (attached at C-6) are all situated at the smaller, so-called, primary side of the molecule, whereas the secondary hydroxyl groups (positioned at C-2 and C-3 of the glucose units) are located at the more open secondary side Since all hydroxyl groups are directed outwards, CDs are very soluble in water (Table 1 1) The CD cavity is shaped by the carbon atoms of the glucose units and the glycosidic oxygen atoms This

configuration makes the cavity relatively apolar which leads to the complexation of apolar substrates in aqueous solution as will be explained in Section 1 4

Table 1.1 *Physical properties of cyclodextrins* [a]

	Glucose-units	Molecular weight	Water solubility (g x l [1])	Cavity dimensions (Å) Internal diameter	Cavity dimensions (Å) Height of cavity
α-CD	6	972	145	4 7-5 3	7 9
β-CD	7	1135	18 5	6 0-6 5	7 9
γ-CD	8	1297	232	7 5-8 3	7 9

[a] See ref 13

According to the crystal structures, the hydroxyl groups at the secondary side of a CD are able to form a network of intramolecular hydrogen bonds (see Figure 1 4) [27,28]

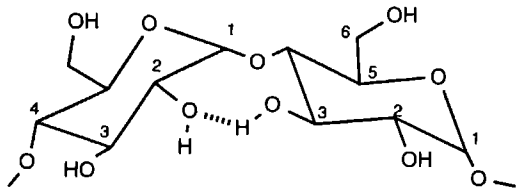


Figure 1.4 *Hydrogen bond formation between adjacent glucose units in a CD*

^{1}H-NMR studies have shown that these hydrogen bonds are also retained in DMSO-d6 solution [29,30] Since DMSO is known to break hydrogen bonds, these experiments suggest that the intramolecular hydrogen bonding network is very strong and probably also present in aqueous solution In α-CD the belt of hydrogen bonds is incomplete because one of the glucopyranose units is in a distorted position so that only four of the six possible hydrogen bridges can be formed [32] In β-CD, the belt of hydrogen bonds is complete, which further stabilises the rigid structure of the cavity of this molecule It was shown in the solid state that the intramolecular hydrogen bonds of β-CD are involved in a flip-flop mechanism in which there is a fast equilibrium between 2-O*H* O-3 and 2-O *H*O-3 [28] Using temperature-dependent NMR studies it was established that in solution the 3 OH of β-CD is the predominant hydrogen donor [31,30] The difference in hydrogen bonding pattern between α- and β-CD results in a different solubility of these compounds in water In γ-CD there is also a

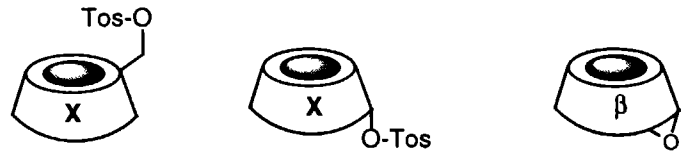
complete belt of hydrogen bonds, but the cavity of this molecule remains rather flexible [33] This probably explains why the solubility of γ-CD in water is higher than that of α- and β-CD (see Table 1 1) Coleman et al have suggested that the formation of aggregates is one of the reasons why CDs have a different solubility [34] No proof, however, was given supporting this explanation

As discussed above, the hydroxyl groups in a CD molecule can be divided into three sets the primary OH groups at C-6, the secondary hydroxyl groups at C-2 and the OH groups at C-3 The C-6 OH groups are the most nucleophilic ones Their pK_a-values are similar to those of other primary hydroxyl groups and amount to 15-16 The most acidic secondary hydroxyl groups are located at C-2 and have a pK_a of 12 1 [35] The higher acidity of the C-2 OH group as compared to the C-3 OH group can be attributed to the hydrogen bond between C-3 OH and the C-2 OH, which facilitates the formation of the oxyanion at C-2 Also the electron-withdrawing acetal function at the β-position of C-2 decreases the pK_a-value of the 2-OH group, causing the C-3 OH groups to be the least reactive groups [36] These differences in properties between the sets of hydroxyl groups can be used in the selective derivatisation of CDs, as will be described in more detail in the following section

1.4 Modification of cyclodextrins

A large number of publications has appeared on the synthesis of new derivatives of CDs For example, in a review article written in 1983 already more than 280 different derivatives were described [37] and many papers dealing with CDs were still to come (see Figure 1 1) In the following paragraphs some general principles used in the modification of cyclodextrins, will be discussed together with some illustrative and interesting examples

Nearly every synthesis of a CD derivative makes use of the difference in reactivity between the three sets of OH-groups present in the CD molecules The relatively high nucleophilicity of the primary hydroxyl groups at C-6 was taken advantage of in the synthesis of the mono-tosylates **1a-c** [38,39,40] These compounds were obtained by reaction of the corresponding CDs with tosyl chloride in pyridine at low temperatures Under these conditions only tosylation at C-6 occurred [41] The formation of di-, tri- and higher substituted CDs could be suppressed by using short reaction times and a large excess of CD Performing the tosylation reaction in aqueous media at pH 13 resulted in the case of β-CD in the formation of **1b** whereas in the case of α-CD the C-2 tosylated product **2a** was obtained [42] The latter cyclodextrin derivative and the β-CD analogue **2b** can also be prepared by an acyl transfer reaction using 3-nitrophenyl tosylate [42,43]



1 **a**: x=α
b: x=β
c: x=γ

2 **a**: x=α
b: x=β

3

Another route to compound **2b** is deprotonation of the (most acidic) C-2 OH groups with NaH in DMF followed by reaction with tosyl chloride.[35] Compounds **1** are frequently used as building blocks since they can easily be reacted with a great variety of nucleophiles (e.g. amines and sulfides) to give new mono-functionalised products directly. The tosyl groups in compounds **2** cannot be directly substituted via an S_N2-reaction, as the nucleophile has to attack from inside the cavity which causes steric hindrance. Also the substitution of the 2-sulphonyloxy-group in α-D-glucose itself is known to be nearly impossible using charged nucleophiles.[44] Compounds **2**, therefore, have to be transformed first into the mono-epoxide under basic conditions. This reaction occurs via an intramolecular reaction of the C-3 OH group attached to the same glucose unit yielding compound **3**. ^{1}H-NMR studies have shown that the *manno*-epoxide is formed under these conditions. Further functionalisation can be achieved by opening this epoxide with e.g. a thiol group. This opening takes place diaxial as can be expected for *manno*-epoxides.[45] The new functional group is now located at the C-3 position and the glucose moiety is converted into an altrose unit (Figure 1.5). This altrose unit is

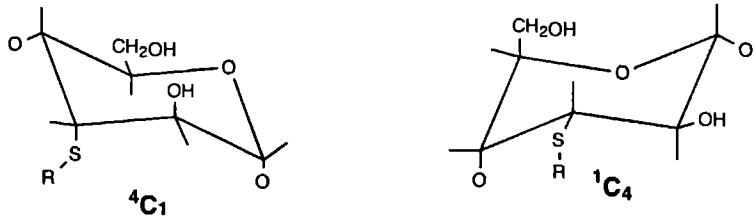


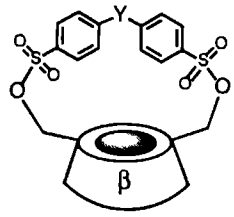
Figure 1.5 *The two conformations of an altrose unit.*

present in the ^{1}C$_4$-conformation as could be concluded from the same ^{1}H-NMR-studies.[46] When ammonia is used to open epoxide **3**, 3-amino-3-deoxy-β-CD is obtained.[47] An interesting alternative synthetic route towards a mono-amino-functionalised β-CD was recently described. The glycosidic bond of a peracetylated α-CD was cleaved and the two end groups of the resulting hexasaccharide were modified. Coupling of this modified compound with a

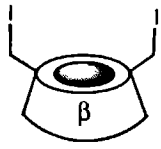
D-glucosamine precursor yielded a heptasaccharide which after ring-closure and subsequent deacetylation gave 2-amino-2-deoxy-β-CD.[48]

The introduction of two functional groups at the secondary side of a CD is difficult since a set of regioisomers will be obtained under most reaction conditions. Fujita et al. synthesised β-CDs containing two tosyl-groups located at C-2 positions.[49] The regioisomers were separated using reversed phase HPLC and transformed to the corresponding di-epoxides. These latter compounds were treated with ammonia to give the three regioisomeric $3^A,3^B$-, $3^A,3^C$- and $3^A,3^D$-functionalised cyclodextrins.[49] Combination of the epoxide opening reaction with the synthetic route that leads to compound **1a** allowed the synthesis of the full set of $3^A,6^X$-di-O-sulfonylated α-CD derivatives.[50] These compounds, however, have as yet not found applications probably because the yields of the reactions were very low.

Other rather thoroughly studied bis-functionalised CDs are the "capped" compounds **4**.



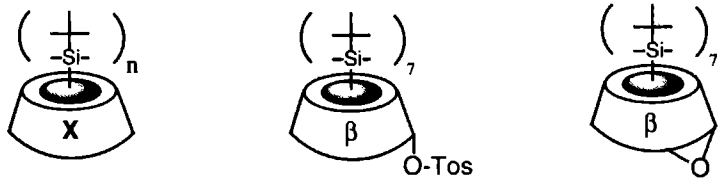
4 **a** = A,B
b = A,C
c = A,D



5 **a** = A,B
b = A,C
c = A,D

They are obtained by reaction of β-CD with bis-sulphonyl chloride compounds of type $Y(C_6H_4SO_2Cl)_2$, in which Y is C=O, CH_2 etc.. The latter reagents can only react with two primary hydroxyl groups if they are located at a certain distance from each other. By changing the Y-part in the structure of the capping reagent it is possible to control the type of regio-isomer that is obtained.[51-56] These capped molecules have been used as building blocks to synthesise new CD derivatives which were used as enzyme mimics.[54-59] In many cases compounds **4** were first converted into the corresponding diiodides **5a-c**, which are more reactive toward nucleophiles. Another synthetic route towards 6^A-6^X-difunctionalised CDs was recently described by Fujita et al.. They introduced one leaving group at the A-position and another one with *different* reactivity at the B, C or D position of the β-CD ring. After separation and purification of the regioisomers, these compounds could be used in selective substitution reactions.[60]

The perfunctionalisation of a set of hydroxyl groups has been used to enhance the solubility of CDs in water [61] and in organic solvents.[62-64] Another reason for perfunctionalisation can be the protection of one set of hydroxyl groups. An important example used in this thesis, is the class of compounds **6a-c**, which contain *tert*-butyldimethylsilyl substituents.[65-67]



6 **a:** x=α, n=6
b: x=β, n=7
c: x=γ, n=8

7

8

These compounds can be obtained in pure form and in high yields (>75%) after flash chromatography. The advantage of the *tert*-butyldimethylsilyl protective group is its high chemical and thermal stability under a variety of reaction conditions while it can be removed easily using very mild conditions.[68] Pregel and Buncel used compound **6b** in the synthesis of the mono-functionalised CD **7** and the monoepoxide **8**.[69] The silyl protected CD is less polar than the parent β-CD, making this compound soluble in organic solvents and allowing it to be purified by chromatography on silica gel. This allows the synthesis and easy purification of new CD derivatives on a larger scale. Several articles have recently appeared in which compounds **6** were used as starting materials for CDs which were peralkylated or peracetylated at their secondary sides.[65-67,70-72] Also the per-(2,3-epoxide) of compound **6b** has recently been described.[73] Compounds **6**,**7** and **8** appear to be very promising starting materials for new CD derivatives as will be shown in this thesis. At the start of this research program no other mono-functionalised silylated CDs than **7** and **8** had been described in the literature.

1.5 Complexation behaviour of cyclodextrins

One of the most important features of cyclodextrins is their ability to form inclusion complexes with various organic compounds, in which the guest molecule is located inside the cavity of the cyclodextrin. On an industrial scale, this property is used to purify α-CD, β-CD and γ-CD by selective precipitation of their inclusion complexes with 1-decanol, toluene and cyclohexadec-8-en-1-one, respectively.[15] Other guest molecules that can be included in the cavities of CDs are polar compounds like acids, amines, small anions (I^-, ClO_4^-),[74] metal-ions,[75] and apolar aliphatic and aromatic hydrocarbons. These inclusion phenomena are usually observed in

aqueous media Complex formation also takes place in DMSO and DMF [76] In the latter case, however, it was concluded from a thermodynamic study that the guest molecule is not always located *inside* the cavity but that it also can be coordinated *over* the cavity, as a lid [77] The requirements and driving forces for the formation of a stable complex are described in this section

The minimum requirement for the formation of an inclusion complex is that the guest molecule must fit partially or entirely into the CD cavity This condition is sometimes forgotten, e g in the claim of a sensor device for pentachlorophenol The latter molecule has a diameter of 9-10 Å and was reported to be included in the cavity of a β-CD derivative which has a diameter of only 6-6 5 Å [78] If guest molecules are too small to fit tightly within a CD cavity no stable complexes can be formed either The usual complex stoichiometry is 1 1 but if a guest molecule is too large to be included by one CD molecule, other complexes with different host-guest stoichiometries can be formed as well like 2 1,[79] 2 2,[80] 3 2,[81] 4 5 [82] and others Also more than one guest molecule can be complexed in a CD cavity leading to 1 2 (host guest) complexes [83] or to three component complexes [84 85]

An important driving force for complexation is the hydrophobic interaction Hydrophobic molecules, or hydrophobic parts of molecules, show higher affinities for CDs than hydrophilic molecules This is a result of the fact that the hydration energy required for the host-guest complex as compared to the hydration energy of the separate host and (apolar) guest molecules is lower when the guest is hydrophobic than when this molecule is hydrophilic [86] The hydration shell around the guest molecule is displaced upon binding in the cavity This freeing of solvent molecules facilitates the complexation and is known as the hydrophobic effect This effect increases when H_2O is replaced by D_2O which leads to an increase in the stability of the complex [87] Water molecules which are located in the CD cavity cannot form so many hydrogen bonds with one another as in the bulk of the solvent Therefore, they can be regarded as molecules of enhanced energy The formation of an inclusion complex results in the release of these high energy water molecules, resulting in a favourable energy change [88 89] Other factors determining the stability of an inclusion complex are Van der Waals interactions,[86 88 90] hydrogen bond formation between host and guest,[91 92] release of strain in the CD ring due to complex formation,[93] and dipole-dipole interactions between host and guest [94] Although many efforts have been made to determine the relative importance of the various contributions, the exact driving forces for complexation are still not clear [94 95]

As already mentioned, the complexation of guest molecules in CDs is usually studied in aqueous solution Some articles, however, have been published describing the complexation in an organic solvent For example, a peralkylated β-CD has been reported which formed complexes with *p*-nitrophenol in heptane [63] The driving force for this complexation was the fact that both the cavity of the CD derivative and the guest molecule were more *polar* than the

solvent. A permethylated β-CD has been shown to bind *p*-chlorophenol in cyclohexane which was concluded to be the result of hydrogen bonding between host and guest.[62] A β-CD derivative, which was peralkylated at the C-6 positions with dodecyl chains linked via amine bonds, has been used for the complexation of *p*-nitrophenol in chloroform. This inclusion complex was stabilised by hydrogen bonds and by the formation of salt bridges between the host and the guest.[64]

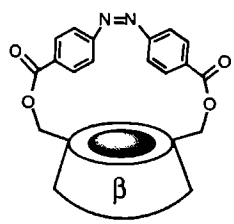
The ability of CDs to form inclusion complexes with a great variety of guest molecules is the basis of their use in supramolecular systems which will be discussed in the next section.

1.6 Cyclodextrins in supramolecular chemistry

Four types of supramolecular structures will be described: host-guest complexes, self-assembled structures, catalytically active structures and rotaxanes.

Host-guest complexes

Since every inclusion complex between a cyclodextrin and a guest molecule in fact represents a supramolecular structure, and a large number of such structures have been described in the literature [13,96], we only will focus on some remarkable examples. The CD derivatives **9** and **10** contain a cap with an azobenzene moiety, which can be switched from the trans-form to the



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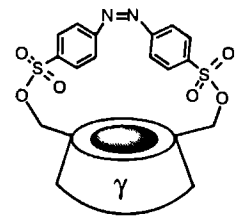

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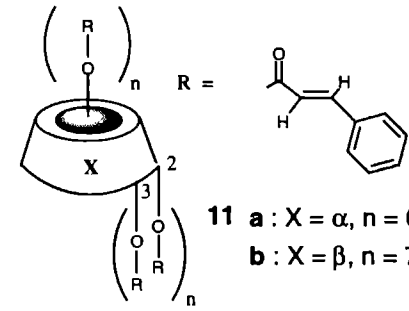


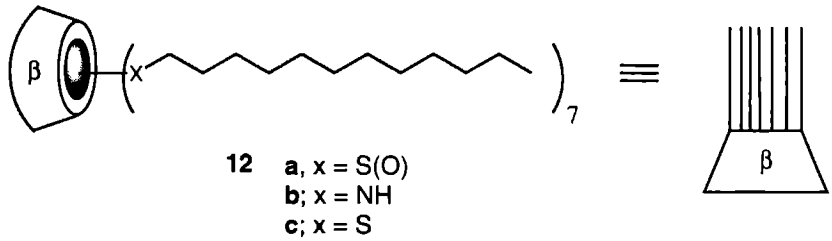
11 **a** : X = α, n = 6
b : X = β, n = 7

cis-form by UV-irradiation (330 nm). The reverse isomerisation reaction can be induced by heating or irradiation with visible light. The cis-isomer of compound **9** showed higher affinities towards guest molecules than the trans-isomer, which was explained by an enlarging of the hydrophobic cavity of the CD as a result of the trans to cis isomerisation.[97] In the case of cyclodextrin **10** a decrease in the affinity towards guest molecules was observed upon the trans to cis isomerisation. This opposite behaviour is due to the formation of a stable self-inclusion

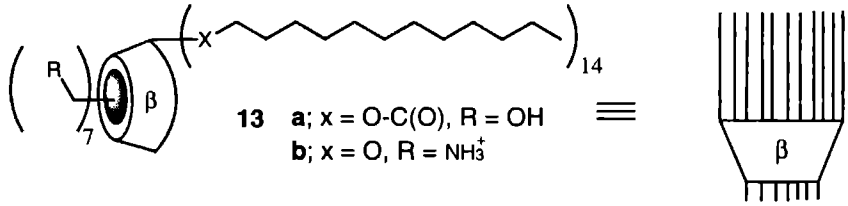
complex between the CD ring and the cis-azobenzene unit which is not possible for the trans form.[98] Molecules of type **9** and **10** may be used as photochemical switches Other examples of such hosts are compounds **11a** and **11b**, which can be obtained by percinnamoylation of α- and β-CD. Upon irradiation, (300 nm) the side groups of **11** form intramolecular cyclobutane bridges,[99] which results in the closure of the CD cavity. N-Methylpyrrolidin-2-one (NMP), which was the solvent used in the cycloaddition reaction, was entrapped in the cavity leading to a so-called carceplex [100] The NMP molecule could be liberated from the carceplex by opening of the cavity with UV-irradiation (254 nm)

Self-assembled structures.

Cyclodextrins modified with long alkyl chains, e g **12**, have been used to prepare monolayers at the air-water interface as is schematically drawn in Figure 1 6 a [101,102]



12 **a**, x = S(O)
b; x = NH
c; x = S



13 **a**; x = O-C(O), R = OH
b; x = O, R = NH_3^+

Compounds **13a-b** [103, 70] have been reported to form vesicles in THF solution At the air-water interface molecules of **13** gave organised structures of the type depicted in Figure 1 6 b [104] The area occupied by molecules **12a-c** at the air-water interface was in good agreement with the external diameter of the CD [101] Guest molecules like azobenzenes could still be included inside the cavities of the CD units without disturbing the monolayers [102 105] Also the photo-induced cis to trans isomerisation of an included compound was still possible.[106] As the CD layers can also be deposited on glass slides,[101] this opens the possibility of using these materials for the development of an information storage device.

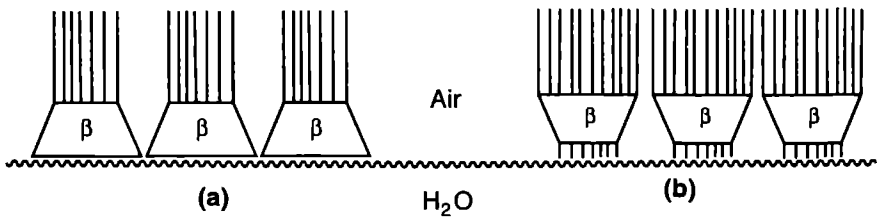


Figure 1.6 *Arrangement of molecules of compounds 12 at the air water interface (a). Idem for molecules of compounds 13 (b).*

It was shown recently that the presence of a long alkyl chain is not always required to give stable CD monolayers: compounds **1** also formed stable monolayers when deposited at the air-water interface.[107]

A CD derivative has been synthesised in which all primary hydroxyl groups are replaced by thiol groups. This compound formed monolayers when it was adsorbed on a gold surface.[108] A voltammetric study showed that the monolayer exhibited high binding affinities for ferrocene and *m*-toluic acid molecules. The relative binding affinities correlated well with those measured in solution.

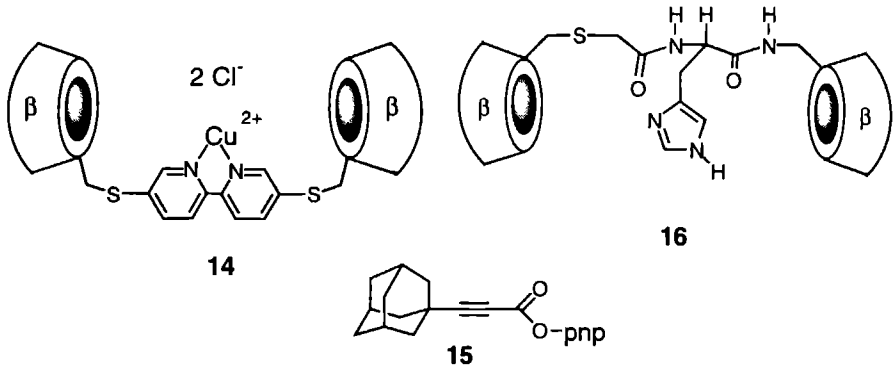
Catalysis

Since CDs have been known for a long time, it is not surprising that they were the first compounds to be used as supramolecular catalysts.[109] Cramer showed in 1953 that the oxidation of feroin was accelerated by β-CD.[12] Since then, many researchers have investigated CDs as catalysts in a large variety of reactions. For example, unmodified CDs have been reported to catalyse the hydrolysis of esters and amides, acyl transfer reactions, oxidation, substitution, elimination and isomerisation reactions.[8,110-112] The inclusion of aromatic compounds followed by an aromatic substitution reaction leads to the regioselective formation of para-substituted compounds in the case of phenolic substrates [113] and of 2,6-disubstituted products when the starting compound is 2-naphthalenecarboxylic acid.[114] The chirality of the CD cavity has led to the use of CDs in asymmetric reactions like reductions, Diels-Alder reactions and numerous oxidation reactions.[115]

Particularly the ester cleavage by CDs has been extensively investigated. For example, accelerations of 6 x 10^6 for the hydrolysis of ferrocene containing esters at pH 10 have been reported.[116] The difference in the ester cleavage rate between two enantiomers (with the same binding affinities) was found to be 62, indicating that the binding geometry of the complex determines the rate of the reaction.[116] CD derivatives that are active as catalysts in ester

cleavage reactions are often referred to as mimics for the enzyme chymotrypsin.[117] The biological function of this enzyme is mainly to cleave amide bonds although it is also able to cleave ester bonds. Menger sceptically noted that most esters investigated in the chymotrypsin model, are *p*-nitrophenol esters which are very easily cleaved, which makes the comparison with chymotrypsin less realistic.[118]

An example of a CD dimer which was used for the cleavage of esters is compound **14** (Chart 1.1).



14 **16** **15**

Chart 1.1

The hydrolysis of ester **15** by complex **14** at neutral pH occurred 10^4 times faster than the uncatalysed reaction.[119]

Another recently developed catalyst is dimer **16**. This compound is moderately efficient in catalysing the hydrolysis of some *p*-nitrophenyl esters of long chain fatty acids.[120]

Rotaxanes

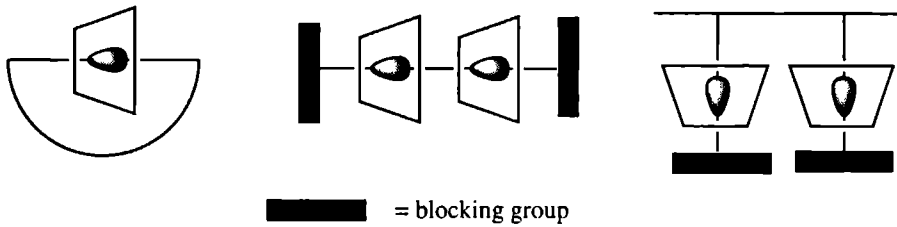
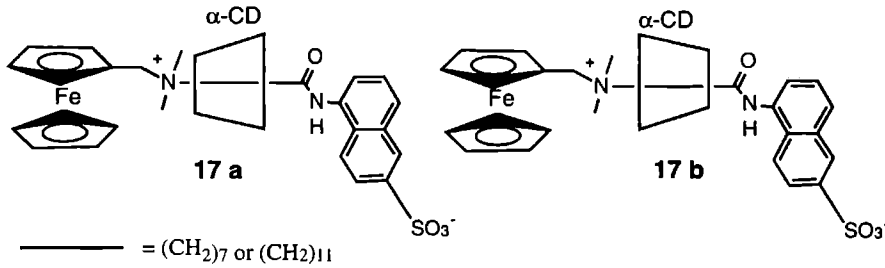
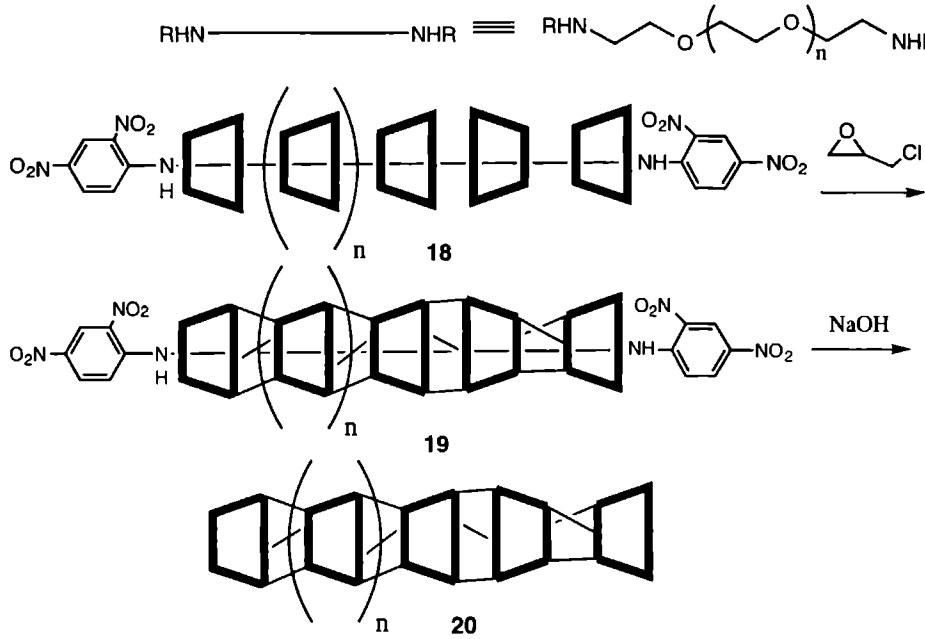


Figure 1.7 *Schematic drawing of catenanes (A) and rotaxanes (B and C) prepared from cyclodextrins.*

Cyclodextrins are currently receiving great interest as building blocks to construct catenanes and rotaxanes (Fig. 1.7).[121,122-125] An example is compound **17** which is an asymmetric [2]rotaxane.[126] The two isomers **17a** and **17b** could be separated and it was shown, surprisingly, that the latter isomer slowly dissociated into α-CD and the dumbbell molecule whereas the former isomer was stable.[121]



A nice example of a polyrotaxane is compound **18**, which was prepared by threading of a large number of α-CDs onto a poly(ethylene glycol) chain. The chain contained two amino-functionalities at both ends which were reacted with 2,4-dinitro-fluorobenzene to give the stoppered compound **18**.[127] The reaction of this polyrotaxane with epichlorohydrin resulted in a crosslinking of the CD moieties to give compound **19**. After removal of the blocking groups with sodium hydroxide, a molecular tube (**20**) was obtained with a molecular weight of approximately 20,000, which corresponds to circa 15 linked CD units.[128]



1.7 Outline of this thesis

This thesis describes new synthetic routes towards monofunctionalised cyclodextrin derivatives and the application of these compounds in several supramolecular systems Every chapter starts with a literature survey on the relevant topic Mono-functionalised CDs can be applied to synthesise CD dimers as is described in Chapters 2 and 3 Some of these dimers were connected via a long alkyl spacer and showed interesting NMR spectra which indicated that the spacer is included in one of the CD cavities These compounds have been studied using ^{1}H- and ^{13}C-NMR spectroscopy (Chapter 2) For one of these dimers a nearly complete assignment could be made for the cyclodextrin proton signals This assignment confirmed the presence of a self-inclusion complex Chapter 3 describes the development of a catalytic system that can be used for the epoxidation of alkenes This system contains a manganese porphyrin which is encapsulated by a CD dimer The latter molecule has an extra binding site that is used to bind a rhodium metal center This metal can activate the manganese porphyrin with the help of formiate anions In the presence of molecular oxygen, this system is able to catalyse the epoxidation of olefins, e g nerol Since the observed turnover numbers for this epoxidation reaction were very low, the complexation of porphyrins in different CD dimers was studied in more detail (Chapter 4) The results suggest that the type of complex formed is dependent on the nature of the linking spacer In the same chapter also a complexation study of toluidino-naphthalene sulphonates is described These molecules are encapsulated by CD hetero-dimers (build up from one α-CD and one β-CD) in a site-specific way, as could be concluded from fluorescence measurements These binding studies can be interesting for future developments of regioselective catalytic systems

Chapter 5 and 6 deal with the use of CDs as sensor devices for the molecular recognition of organic molecules in water and air, respectively Chapter 5 describes CDs that contain covalently linked fluorescent groups that are encapsulated in the CD cavities and can be displaced by a guest molecule if this latter has a higher binding affinity The changes in the microenvironment of the fluorescent probe that occur upon guest binding are monitored by fluorescence spectroscopy and can be used for the detection of organic molecules like adamantanecarboxylic acid In the last chapter of this thesis (Chapter 6) a sensor based on a piezoquartz microbalance is described This device uses a quartz crystal that is very sensitive towards mass changes if it is coated with a layer that gives an interaction with the analyte to be detected Cyclodextrin derivatives have been used as a coating for these crystals and have been investigated on their ability to detect organic vapours in air

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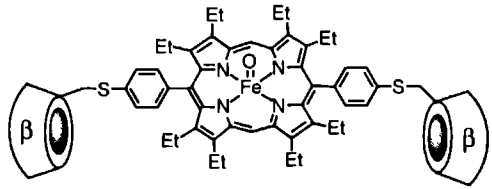
CHAPTER 2

Synthesis and Characterisation of Cyclodextrin Dimers

2.1 Introduction

Cyclodextrins (CDs) are cyclic oligomers of D-glucose which are soluble in water. Because of their doughnut-shaped structure they can bind various types of organic molecules. For this reason cyclodextrins have been used to mimic the binding sites of enzymes. Until now most studies have focused on monomeric CDs in which substrates are bound with relatively low binding constants. In order to obtain systems in which the substrates are held more tightly several research groups have coupled two CD cavities and investigated the binding properties of such dimers. CD dimers can roughly be divided into two groups: CDs that are connected by spacers linked at the primary side of the molecules and CDs that are connected by spacers at the secondary side. In this chapter we describe the synthesis of novel CD dimers belonging to the last class of compounds. In the following sections we will first give an overview of CD dimers reported in the literature. When the whole literature concerning these dimers is considered, it is striking that often very few experimental details on the preparation of the compounds are given. For instance, dimers that have been mentioned in the literature without any synthetic details are compounds **1**, **2**[1] and **3**.[2]



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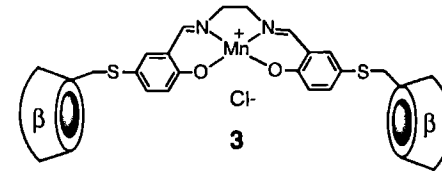

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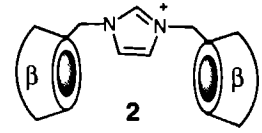
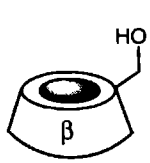


2

2.2 Cyclodextrin dimers connected via their primary sides

The synthesis of CD dimers that are connected via their primary sides can be achieved in the easiest way via a double acylation reaction involving the primary hydroxyl groups of two CDs, e.g. **4**. Using thioxanthone-3,6-dicarbonyl dichloride as the acylating agent, dimer **5** was obtained. This compound showed very high binding affinities for ditopic guest molecules like **6** ($K_b = 7 \times 10^8\ M^{-1}$).[3]



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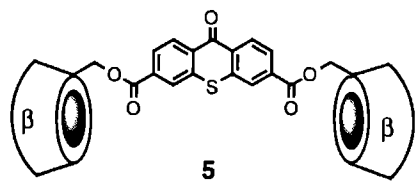

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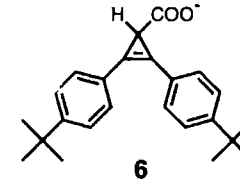
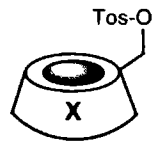
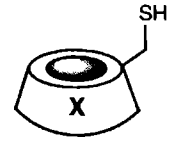


6

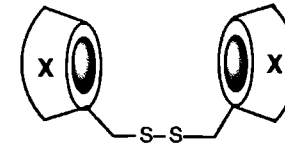
Problems that arise when this acylation method is used, are intramolecular diester formation between the diacid chloride and one CD moiety, the formation of CD dimers that are connected by more than one linker, and the formation of oligomers. Also the possibility of an acylation reaction on the secondary side of the CD cannot be excluded. A different synthetic route, therefore, has been applied for most of the other CD dimers described in the literature. This route always starts with a monotosylation reaction on the primary side of the cyclodextrin to give compounds **7a-7c**.[4-6]



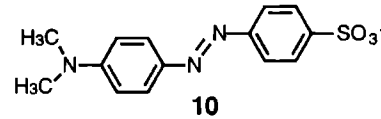
7 **a**: x = α
b: x = β
c: x = γ



8 **a**: x = α
b: x = β
c: x = γ



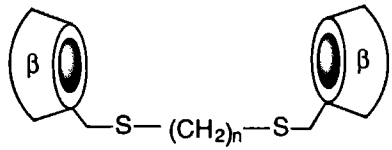
9 **a**: x = α
b: x = β
c: x = γ



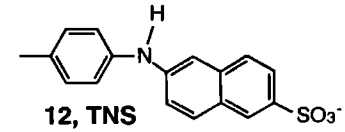
10

These monotosylates can be easily transformed into compounds **8a-c** with the help of thiourea. The latter compounds are useful building blocks for the synthesis of CD dimers since they possess one reactive SH-group in the presence of 20 less reactive hydroxyl groups. For example, dimers **9a-c** can be obtained from **8a-c** by oxidation in air [7,6] or by reaction with

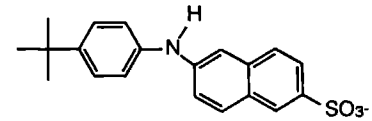
iodine.[8] Dimers **9a-c** were used to complex methyl orange **10** and the measured binding constants were found to be 3-12000 times higher for the dimers than for the monomeric CDs, demonstrating that the two linked CDs act cooperatively in the binding process.
Similar dimers (**11a-e**) were obtained by reaction of α,ω-dimercaptoalkanes with compound **7b**. These dimers were used to investigate the influence of the spacer length on the complexation of large substrates like 2-*p*-toluidinonaphthalene-6-sulphonate (TNS, **12**) and 2-*p*-*tert*-butylanilinonaphthalene-6-sulphonate (BNS, **13**).[8] The highest binding constant was



11 **a**: n = 2 **d**: n = 5
b: n = 3 **e**: n = 6
c: n = 4



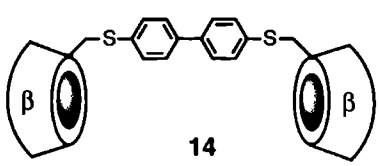
12, TNS



13, BNS

observed for dimer **11a**. If the tether was more than two methylene groups long, the affinity of the dimer for BNS diminished. The binding affinities of dimer **9b** for TNS and BNS were approximately 3 and 100 times lower than those of dimer **11a** for these compounds. This may indicate that there is an optimal tether length for binding in this class of dimers. The gradual decrease in the free energy of binding which was observed on going from **11a** to **11e**, however, may also be the result of a self inclusion of the linking spacer (see our results described in Section 2.5.3).

Dimers **14**[9] and **15a**[10] were synthesised in a similar way as described above for dimers **11a-e**. In this case the monotosylate **7a** was first converted[11] into the monoiodide **16** because the latter derivative is more reactive towards nucleophilic attack by sulfides. As mentioned in Chapter 1 compound **15a** was used to bind Cu(II) or La(III) and was found to catalyse the hydrolysis of esters, e.g. **17-19**.[10,12] The closely related dimer **15b** was synthesised starting from a monohydroxy-β-cyclodextrin derivative, of which all the remaining hydroxyl groups had been methylated. The bipyridyl unit was used to bind a Re(I)-centre.[13]



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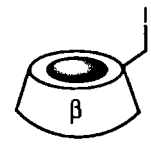

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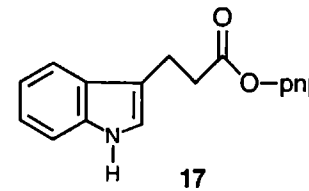

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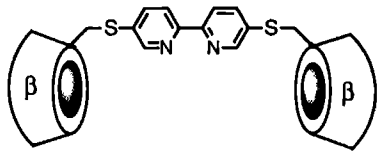
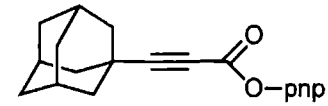


15 **a**: unprotected CD
b: permethylated CD



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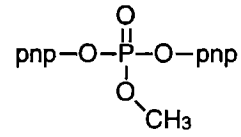
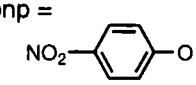
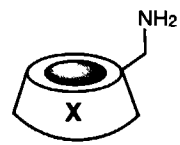


19

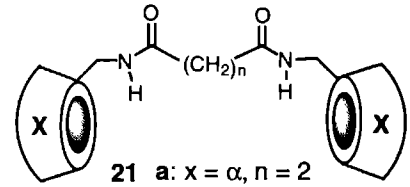
pnp =



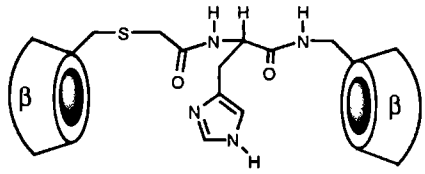
In a different approach towards CD dimers, the tosylate groups of compounds **7a,b** were substituted by azide groups, which were subsequently reduced to yield the monoamino-functionalised cyclodextrins **20a,b**.[4] The active *m*-nitrophenol esters of diacids reacted selectively with the amino functionality of these compounds to give the dimers **21a-c** in good yields (68-94%).[14] In spite of the large amounts (50 g of compound **21b** !) that were obtained, no binding studies or applications of these dimers have been reported so far.



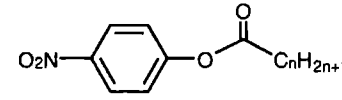
20 **a**: x = α
b: x = β



21 **a**: x = α, n = 2
b: x = β, n = 2
c: x = β, n = 3



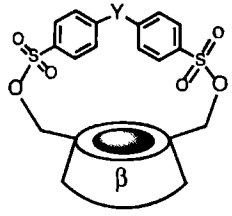
22



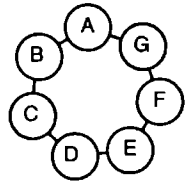
23 n=1,2,5 or 11

A combination of an amidation reaction and a nucleophilic substitution reaction involving molecule **20b** and molecule **16** has been used to synthesise the asymmetric dimer **22**. This dimer was used to catalyse the hydrolysis of esters **23**.[15]

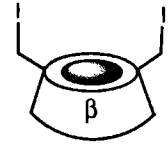
Other examples of primary-side functionalised dimers are the compounds that have been synthesised using the capped cyclodextrins **24a-c** (see also Chapter 1). By using different functionalities (-Y-) the regio-isomers **24a**[16], **24b** and **24c**[17] could be obtained. These compounds were subsequently converted into the diiodo compounds **25a-c**. Reaction of a tetraphenylporphyrin containing four thiol groups with two molecules of **25c** yielded compound **26**.[18] This dimer was used as a catalyst for the epoxidation of alkenes and was found to be more active than the related water soluble tetrakis(*p*-sulphonatophenyl)porphyrin (TsPP).[19] In our opinion the difference in the reported activities are the result of the difference in aggregation behaviour between these two porphyrins, which was not recognised by the authors. The reference porphyrin aggregates under the used conditions (see also Chapter 4) whereas the porphyrin unit in dimer **26** cannot aggregate, resulting in a more effective epoxidation in the latter case. Recently, regioselective and stereoselective epoxidation reactions were reported using dimer **26**. These reactions show that the CD cavity is able to control the position and the face of attack on the alkene.[20] The cavities of dimer **26** have also been used to bind quinone derivatives. This study has provided interesting information on electron transfer



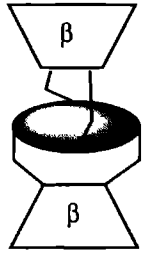
24 **a**: A,B-substituted
b: A,C-substituted
c: A,D-substituted



nomenclature for glucose units in CDs, seen from the primary side.



25 **a**: A,B-substituted
b: A,C-substituted
c: A,D-substituted



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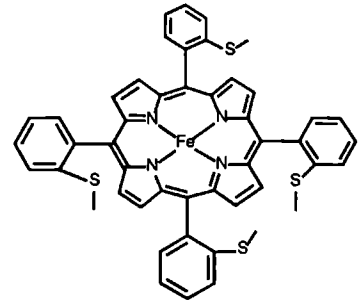
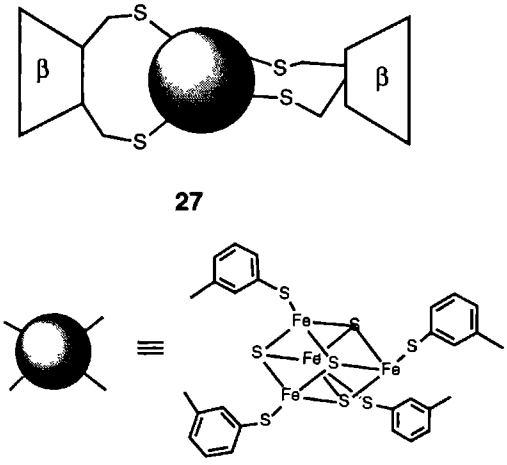


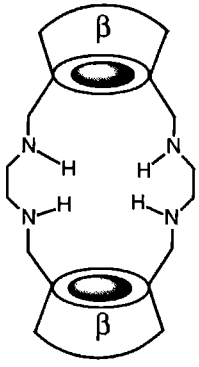
26

reactions between quinones and porphyrins in aqueous solution.[21] Recently the synthesis of a compound similar to **26** was described in which the sulfide bonds are replaced by amide bonds.[22]

Reaction of compound **25c** with an excess of 1,3-dithiophenol followed by ligand exchange with an iron-sulfur cluster afforded dimer **27**.[23] Many synthetic Fe_4S_4-type clusters have been prepared to mimic the active core of ferredoxins. Unlike the natural ferredoxins, these clusters are usually unstable in aqueous solution. Dimer **27**, however, contains an iron-sulfur cluster which is shielded from the aqueous environment by the two cyclodextrins. This makes the cluster about twenty times more stable in water than the same cluster in the absence of the cyclodextrin moieties.[23]

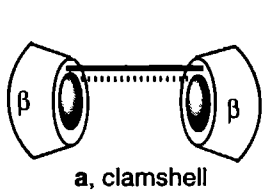


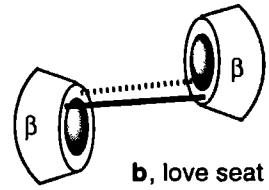
27



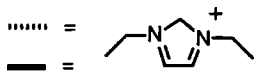
28

Another interesting approach towards the synthesis of CD dimers was developed by Tabushi et al..[24] They succeeded in preparing doubly linked CD dimers, e.g. dimer **28**, in two reaction steps. First a regioisomeric mixture of **24b** and **24c** was reacted with an excess of ethylene diamine. Subsequently the product was treated again with a slight excess of a mixture of **24b** and **24c**. This gave dimer **28** in 16% yield. Compound **28** showed cooperative binding of the substrates TNS and **10**.[24] Several years later Breslow et al. extended Tabushi's procedure by preparing dimer **29** starting from compound **25a**. No synthetic details, however, were mentioned.[25] Compound **29** can be present as two isomers: an occlusive or "clamshell" configuration (**29a**), in which the two CD units act cooperatively in the binding of a substrate, and an aversive or "love seat" configuration (**29b**), in which such a cooperative binding cannot occur. The "clam shell" configuration showed a binding constant $K_b > 10^9\ M^{-1}$ for substrate **6**, whereas the "love seat" displayed a much lower binding constant for this substrate, viz. $K_b = 10^4\ M^{-1}$.[26]

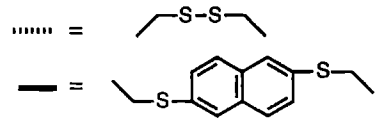




29

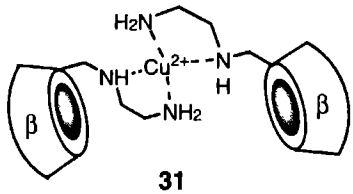


30

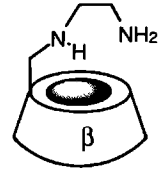


Other doubly-linked CD dimers that have been prepared by Breslow et al.,[27] are compounds **30a** and **b**. These dimers are unique in the sense that they contain two linkers of unequal length. The synthesis was carried out via the A,B-capped CD **24a** which was transformated into the di-iodide **25a**. Subsequent reaction with naphthalene-2,6-dithiol afforded a mixture of dimers which still contained two iodine functionalities. Further substitution of the iodines by potassium thioacetate, followed by hydrolysis, yielded a dithiol. This compound was oxidised with air to yield a mixture of isomers. Two of the possible isomers could be separated by column chromatography. The occlusive isomer **30a** in which the S-S linkage appeared to be A,A' and the naphthalene linkage B,B' was formed as the major product. The other isomer that could be obtained in pure form was **30b** which had the S-S linkage at positions A',B and the naphthalene linkage at positions A,B'. Again the occlusive dimer **30a** showed much stronger affinities for large substrates (K_b up to 10^{10} M^{-1}) than the aversive dimer **30b**, which displayed binding constants that were in the same range as those of β-cyclodextrin itself.[27]

An example of a dimer in which the CD units are connected via the primary sides by non covalent interactions, is compound **31**. The monomeric unit of this dimer (**32**) was synthesised by a nucleophilic substitution reaction between ethylene diamine and molecule **16**. When Cu (II) ions were added to **32**, dimer **31** was formed, which displayed a seven-fold increase in the binding constant of the substrate Brilliant Yellow as compared to molecule **32** itself.[28]



31



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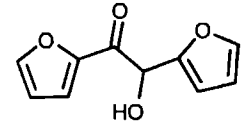
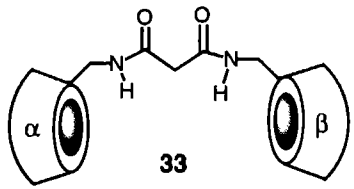


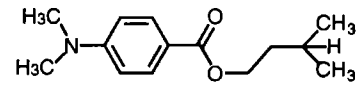
furoin

Dimer **31** was successfully used to enhance (20-fold) the oxidation rate of furoin at pH 10.5 compared to the uncatalysed reaction.[29]

A new type of CD dimer (**33**), a so-called cyclodextrin hetero-dimer, was recently described by Ueno et al..[30] The molecule consists of two CD units of different size and was synthesised by a



33

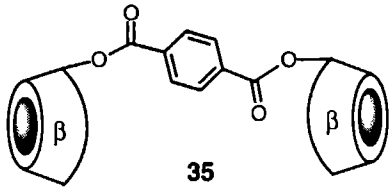


34

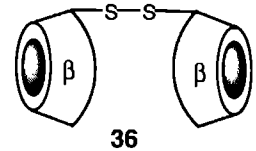
N,N'-dicyclohexylcarbodiimide (DCC) coupling reaction of monoamino-functionalised CD **20a** with singly protected malonic acid. After deprotection and a second DCC coupling with compound **20b** dimer **33** was obtained in 60% overall yield. This dimer is able to bind the substrate isoamyl *p*-dimethylaminobenzoate **34** in a site specific and cooperative way.[30]

2.3 Cyclodextrin dimers connected via their secondary sides

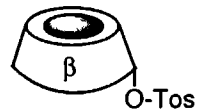
Less examples are known of CD dimers that are connected via a linking spacer at their secondary sides. The first example, dimer **35**, was mentioned incidentally in an article of Breslow.[3] In the same article he described the synthesis of dimer **36**. The latter compound was prepared starting from the monotosyl-functionalised β-cyclodextrin **37**, which was treated with base to give the 2,3 mannoepoxide **38**. Opening of the epoxide with benzylmercaptan followed



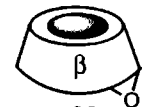
35



36



37

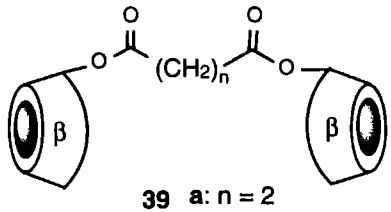


38

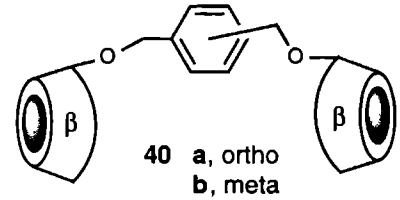
by reduction to the thiol and oxidation in air afforded the disulfide **36**. This dimer showed no enhanced binding affinities for large substrates. An explanation for this negative result might be that the cavities are blocked due to the tight S-S linkage.[3] The most successful (in terms of the achieved cooperative binding) secondary side-linked dimers were described by Harada et al.[31]

They prepared compounds **39a-b** by an esterification reaction of β-CD with bis(*m*-nitrophenyl)dicarboxylates) at room temperature. Dimers **39** showed a 2-4 fold binding enhancement of the fluorescent probe TNS when compared to β-CD.[31]

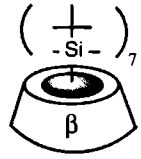
As part of a joint project between the Organic Chemistry Departments of the university of Twente and Nijmegen, van Dienst from the group of Reinhoudt prepared compounds **40a-c** by reacting the corresponding isomer of α,α'-dichloroxylene with the lipophilic silylated β-cyclodextrin **41**. After desilylation, the dimers were obtained in pure form. Using the same procedure also the phenantroline containing dimer **42** could be synthesised.[32]



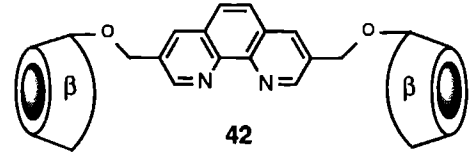
39 **a**: n = 2
b: n = 3



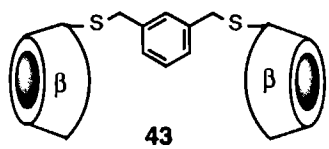
40 **a**, ortho
b, meta
c, para



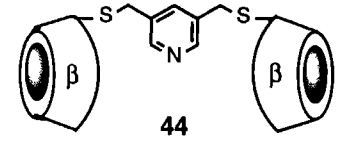
41



42

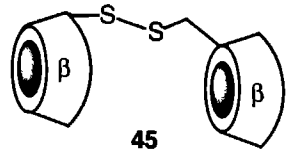


43



44

Very recently, dimers **43** and **44** were prepared by Lawrence et al. [33] from the monoepoxide **38** and bis(mercaptomethyl)benzene and bis(mercaptomethyl)pyridine, respectively. These dimers were characterised by 2D-NMR spectroscopy. It was concluded that the two CD moieties of **43** and **44** were indistinguishable in the ^{1}H-NMR spectrum. NMR experiments showed that the guest molecule **10** can be fully encapsulated by the CD dimers.[33] Metalated macrocycles, e.g. porphyrins, can also be bound in dimers **43** and **44**.[34] It was found that the pyridine unit of **44** can act as a third binding site for the metal centre of the macrocycle, thereby increasing the binding constant by a factor of 10^2-10^4 as compared to dimer **43**.



45

Another new type of dimer is **45** which contains two β-CD units that are covalently attached in a head to tail fashion. This compound is the first example of a third class of dimers, viz.dimers of which the two CDs are connected via different sides. Host molecule **45** showed a three times smaller binding affinity for TNS than the "tail to tail" dimer **36** indicating the importance of the more open secondary side of the CD cavity for the complexation process.[35]

2.4 Synthetic strategy towards pure CD dimers

As described in the previous sections, a large number of cyclodextrin dimers have been prepared. Most of them are connected via the smaller primary sides. There are fewer reports on dimers linked via their secondary sides. These compounds are more interesting because it is believed that the complexation of large guest molecules takes place on this side of the cyclodextrin.[36] Also chiral recognition at the secondary side is more pronounced than on the primary side.[37,38] The reason why most researchers have focused on CD dimers linked via the primary side probably is the easier accessibility of the monofunctionalised compounds **7** as compared to the monofunctionalised compound **37**. Compound **7b** can be purchased since 1994 from "Cyclolab" in Budapest. A severe problem in the modification of CDs is the purification of the reaction products. The usual purification steps are precipitation, crystallisation, ion exchange column chromatography and reversed-phase HPLC. Most of these techniques are scale limiting and do not always guarantee the purity of the compounds. Already in 1978 Lehn mentioned that : "the general accepted characterisation of organic molecules like elemental analysis, TLC, IR, and UV cannot always cope with the multiplicity of products (e.g. positional isomers) that can be formed during the reactions of cyclodextrins".[39] Underestimation of this problem of characterisation has led to reported CD derivatives of which the purities have not been subjected to sufficient critical scrutiny. A good characterisation, according to Lehn, requires a combination of HPLC with ^{1}H- and ^{13}C-NMR spectroscopy. A few years ago Stoddart repeated Lehn's criticism by stating : "many reports have appeared (in the field of CD chemistry) that include highly exaggerated claims of selectivities operating in reactions and of the purities of the cyclodextrin derivatives isolated".[40]

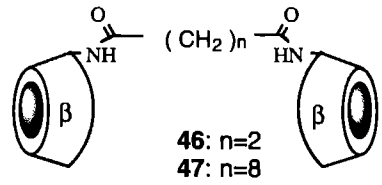
To make sure that the CD dimers which we planned to synthesise consist of well-defined structures and are of a high purity, we have used silylated CD derivatives described for the first time by Fügedi for β- and γ-CD[41] and later by Mitoh et al. for α-CD.[42] These authors used the

tert-butyldimethylsilyl group to protect the primary hydroxyl groups of the cyclodextrin molecule. This strategy has the advantage that the resulting derivatives can be purified by flash chromatography instead of reversed-phase HPLC, which allows larger scale preparation of derivatives. Pregel and Buncel were the first to use these silylated compounds for obtaining monofunctionalised CDs.[43]

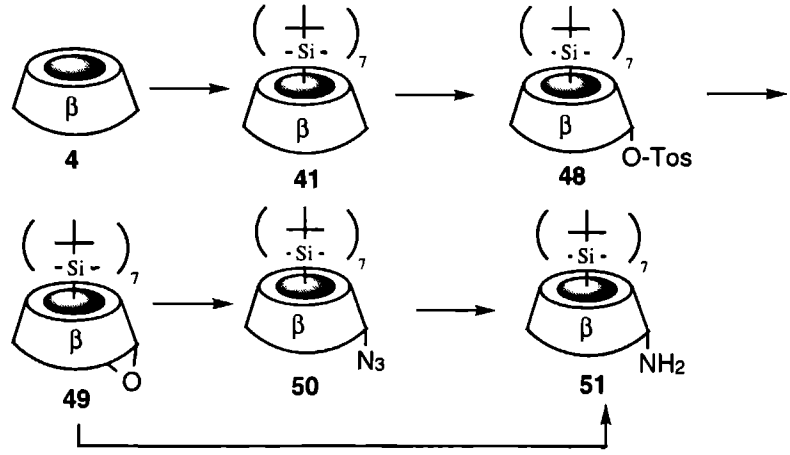
2.5 Results and Discussion

2.5.1 Synthesis of cyclodextrin homo-dimers

We decided to embark on the preparation of two β-CD dimers that are linked by methylene chains of different lengths, viz. compounds **46** and **47**.



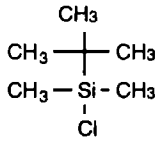
The monofunctionalised CDs that are required for this preparation were synthesised as outlined in Scheme **2.1**.



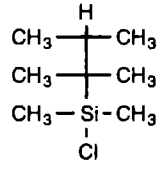
Scheme 2.1

The starting compound, β-cyclodextrin **4**, was silylated as described[43] to give compound **41** in 82% yield after purification by column chromatography. Deprotonation of compound **41** with NaH in refluxing THF, followed by reaction with tosyl chloride yielded a mixture of the starting material and the mono- and di-tosylated products. Since this reaction always leads to a statistical mixture of products a maximum yield of approximately 35% can be expected. The mono(2-O-)tosylate **48** was obtained in 27% yield after column chromatography. A total of 40% of the starting compound could also be recovered in this way. Compound **48** has previously been synthesised via a tosyl transfer reaction under basic conditions, albeit in a lower yield (20%).[43] A direct replacement of a 2-sulphonyloxy group at an α-D-glucose unit by a charged nucleophile is known to be virtually impossible.[44] When, however, an antiperiplanar hydroxyl group at C-3 is present which can be deprotonated, the resulting oxide anion will attack the tosyl group yielding the mannoepoxide.[45] Compound **48**, therefore, was first converted into the epoxide **49** using sodium ethoxide in refluxing dry ethanol (see Scheme 2.1). The ^{1}H-NMR spectrum of this compound confirmed that a manno-epoxide indeed had been formed. If the tosyl group had been situated at the C-3 instead of at the C-2-position this would have resulted in the formation of an allo-epoxide.[43]

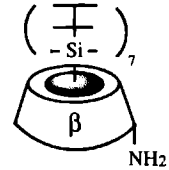
Compound **49** could be converted into **50** by treatment with LiN_3 in refluxing dry ethanol. After reduction of the azide group with $Pd/C/H_2$, compound **51** was obtained (48% yield starting from **48**). Epoxide **49** could also be opened by nucleophilic attack of ammonia on carbon C-3 using an anhydrous, saturated solution of this reagent in ethanol in an autoclave to give the monoamino-cyclodextrin **51** directly. The yield amounted to 83% after column chromatography. Of the two methods, the latter provides a shorter route with higher yield and less purification steps.



tert-butyldimethyl silyl chloride = TBDMS



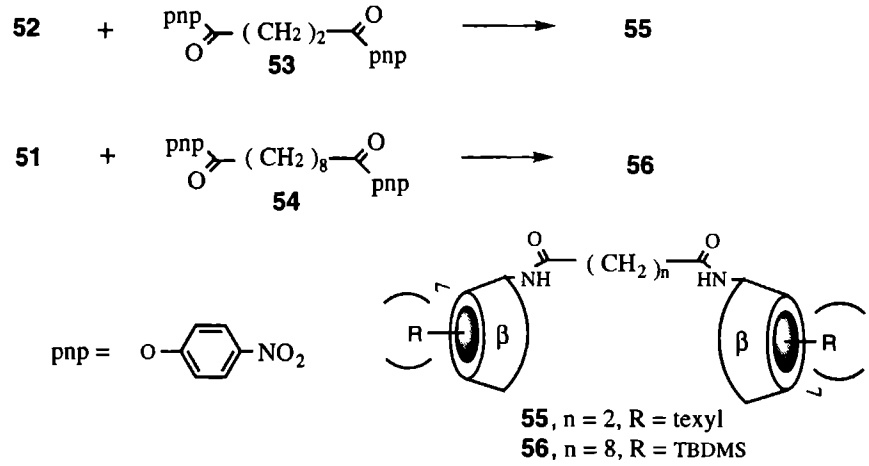
dimethyltexyl silyl chloride = "texyl"



52

Instead of *tert*-butyldimethylsilyl chloride as the protecting group for the primary side of β-CD, we also investigated the use of the texyldimethylsilyl chloride protecting group which is cheaper and easier to handle. Using this group similar yields as for the synthesis of compound **51** were obtained in the preparation of compound **52**. Since a larger excess of the reagent was found to be necessary to drive the reaction with the primary hydroxyl groups to completion we applied compound **52** only to synthese dimer **46**.

Cyclodextrin dimers **55** and **56** were obtained by the reaction of compound **52** or **51** in refluxing THF with the bis-4-nitrophenyl esters of succinic and sebacic acid (Scheme 2 2) Purification by column chromatography yielded **55** and **56** in 76% and 57% yield, respectively Desilylation of the products was achieved with tetrabutylammonium fluoride



55, n = 2, R = texyl
56, n = 8, R = TBDMS

Scheme 2.2

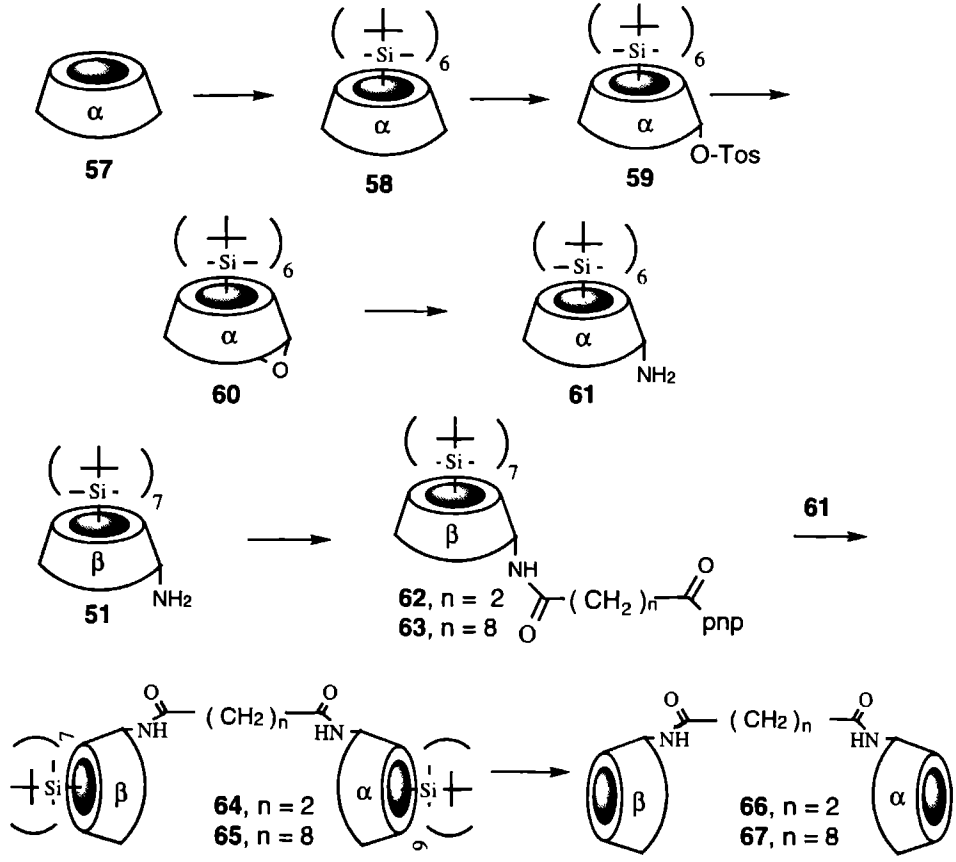
(TBAF) in refluxing THF This afforded **46** and **47** in good yields (95 and 88%, respectively) after workup These compounds were characterised by elemental analyses and spectroscopic methods The NMR spectrum of compound **47** will be described in more detail in Section 2 5 3

2.5.2 Synthesis of cyclodextrin hetero-dimers

A challenging type of CD dimer is the hetero-dimer, consisting of two CD units of different size, e g α-CD and β-CD If we would be able to bind a substrate in such a dimer in a site specific way, this would open up the possibility to perform regioselective and/or enantioselective reactions The binding of organic molecules by CD hetero-dimers will be discussed in more detail in Chapter 4

The synthetic route which we followed to obtain CD hetero-dimers is given in Scheme 2 3 First α-cyclodextrin **58** was prepared as described above for the corresponding β-cyclodextrin **41**, except that the solvent used in the silylation reaction was THF/pyridine instead of pure pyridine to avoid precipitation of reaction intermediates (i e compounds with less than six silyl

groups). The yield of **58** amounted to 69%. The use of DMF as the solvent and imidazole as the base was reported by Takeo et al. to give similar yields of compound **58**.[42] This silylated compound was transformed into the monoamino functionalised CD **61** in an analogous way as described above for compound **51** with comparable yields. The cyclodextrins **62** and **63** containing an active ester were obtained by reaction of compound **51** in refluxing THF with a tenfold excess of the bis-4-nitrophenyl (pnp) esters of the appropriate dicarboxylic acids.



Scheme 2.3

Purification by column chromatography gave **62** and **63** in 51 % and 63 % yield, respectively. This purification was found to lead to partial decomposition of the compounds, probably because of a self-catalysed hydrolysis of the active ester or because of the occurrence of an acyl transfer to one of the secondary hydroxyl groups of the CDs.[46] The decomposition could be minimised by reducing the volume of the column and by using higher flow rates. Because of their instability compounds **62** and **63** were treated as soon as possible with the

monofunctionalised α-CD **61** in refluxing THF, yielding the corresponding dimers **64** (61 %) and **65** (80 %). Desilylation of these products was achieved with tetrabutylammonium fluoride in refluxing THF. The compounds were subsequently dissolved in a small volume of water and precipitated by addition of acetone. After repeating this precipitation twice compounds **66** and **67** were obtained in 50 and 71% yield, respectively. The structures of these compounds were fully established by elemental analyses and spectroscopic methods. The interesting NMR spectra of compound **67** will be described in more detail in the next section.

2.5.3 NMR study on the conformational behaviour of CD dimers

The characterisation of the dimers described in Section 2.5.1 and 2.5.2 by NMR spectroscopy afforded some interesting features. The NMR spectra of the symmetric dimer **47** in DMSO-d6 (Fig. 2.1.a) and in D_2O (Fig. 2.1.b) will be discussed first.

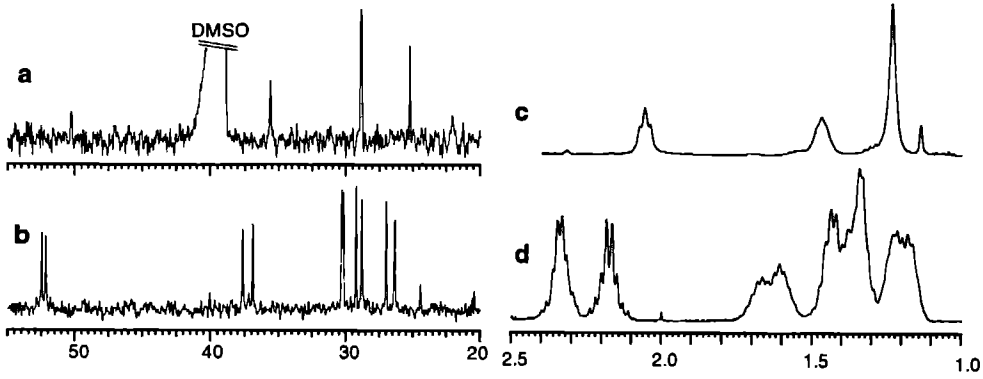


Figure 2.1 *^{13}C-NMR of compound 47 in (a) DMSO-d6 and (b) D_2O and ^1H-NMR spectra in (c) DMSO-d6 and (d) D_2O.*

In the ^{13}C-NMR spectrum of compound **47** in D_2O four signals for the octamethylene spacer were expected as a result of the symmetry in the molecule (Fig. 2.2 conformation a). Eight distinct signals, however, were observed in the region of 20-40 ppm (see Fig. 2.1.b). Also the CD-carbon atom, which resonates at 52 ppm (C-3', next to the amide bond), and the carbonyl atom of the spacer (177 ppm, not shown) appeared as two signals instead of one. The proton spectrum of **47** also showed twice as many signals as expected for the spacer (Fig. 2.1.d). We propose that this doubling of signals is the result of the presence of two identical conformations in which the alkyl chain is complexed by either one of the cavities as is shown in Fig. 2.2.b. The complexation of alkyl chains by cyclodextrins is known to be an energetically favourable proces.[47] In an attempt to break down this self-inclusion complex we increased the temperature

gradually up to 90 °C. Also an equal volume of DMSO-d6 was added to the D_2O solution to break any possible hydrogen bonds. Neither of these experiments resulted in any changes in the proton spectrum. We therefore can conclude that the conformation in which an alkyl chain is bound in the CD cavity is very stable. When, however, the compound was dissolved in pure DMSO-d6 both the ^{13}C- and the ^{1}H-NMR spectra (Figs. 2.1.a and 2.1.c) were simplified. Apparently, the spacer is released in pure DMSO and as a result a symmetric structure (Fig. 2.2.a) is obtained.

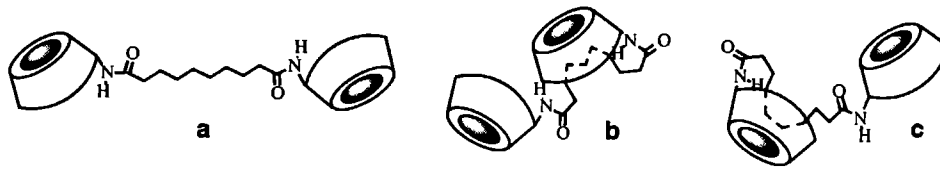


Figure 2.2: *Possible conformations of cyclodextrin dimers in solution.*

The ^{13}C-NMR spectrum of the asymmetric CD hetero-dimer **67** also appeared to be more complex in D_2O than in DMSO-d6 (see Figs. 2.3.a and 2.3.b).

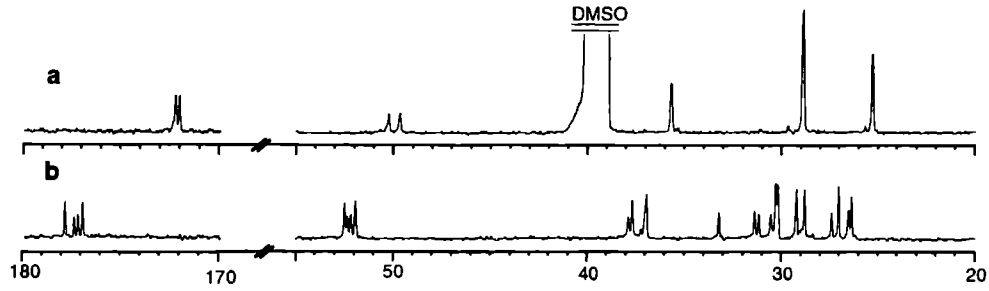


Figure 2.3 *^{13}C-NMR spectra of compound 67 in (a) DMSO-d6 and (b) D_2O*

The spectrum in D_2O solution showed sixteen signals for the octamethylene spacer in the 20-40 ppm region. In addition, four signals were observed for the carbon atoms at C-3' and four signals for the carbonyl carbon atoms in the spacer (Fig. 2.3.b). This multitude of signals can be explained by assuming that the spacer is bound either in an α-CD or in a β-CD cavity which results in two conformations (Fig. 2.2 conformations b and c) which do not exchange on the NMR time-scale. Since the dimer is asymmetric these two conformations are not equivalent, resulting in a doubling of the signals observed for compound **67**. The ^{13}C-NMR spectrum of **67** in DMSO-d6 (Fig. 2.3.a) is simple, as it was in the case of **47** in DMSO-d6, and in accordance with the structure shown in Fig. 2.2.a. The two signals that remain for both the carbon atoms at C-3' and for the carbonyl atoms of the spacer are the result of the asymmetry in

the hetero-dimer In the ^{13}C-NMR spectrum in D_2O all signals for identical carbon atoms appeared in an equal ratio, viz 2 1, supporting the presence of two conformers The fact that this ratio is observed for all pairs of carbon atoms indicates that relaxation effects can be ignored The two carbonyl signals with the highest intensity, at 176 9 and 177 8 ppm, in the ^{13}C-spectrum of **67** in D_2O resonate at exactly the same frequency as the carbonyl signals of compound **47** in D_2O (spectra not shown) We therefore ascribe these two signals to the conformation in which the alkyl chain is bound in the β-CD unit Given this assignment we may conclude that the spacer of compound **67** is approximately 2/3 of the time bound by the β-CD unit and 1/3 of the time by the α-CD unit

A detailed assignment of most of the hydrogen atoms of compound **47** was achieved by a systematic ^1H-NMR study The full details of this assignment will be described elsewhere [48] Briefly, for the attribution of the CD resonances, the anomeric protons (H-1) were used as starting points The magnetisation was transferred through scalar coupling to the non-anomeric protons of each glucose unit using COSY [49], RELAYs [50] and HOHAHA [51] experiments The

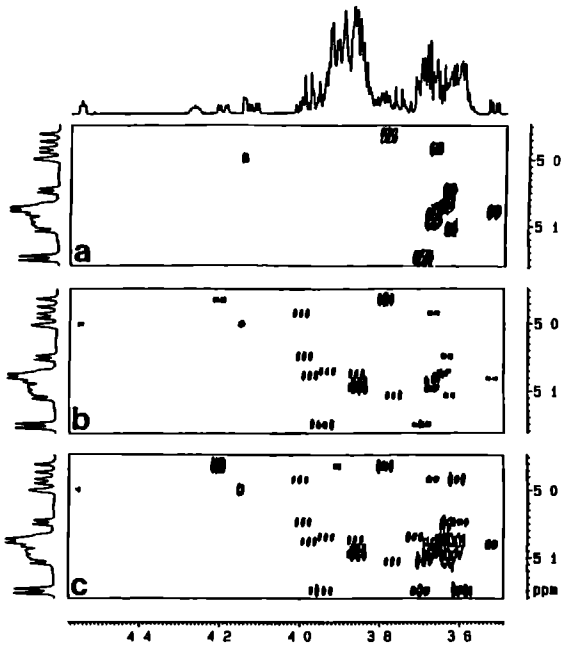


Figure 2.4 *600 MHz 2D semi-soft ^1H-NMR spectra of compound 47 in D_2O, a) COSY, b) single RELAY decoupled in F1, c) double RELAY decoupled in F1*

resolution of these spectra could be improved using "semi-soft" experiments [52] which allowed the intraresidue assignment Some of these spectra are shown in figure 2 4 which are illustrative for the high resolution obtained with these methods

Using NOESY [53] experiments the adjacent H-1 and H-4's could be found via dipolar interactions and therefore a full sequencing of the CD units was possible In figure 2 5 a classical NOESY spectrum of compound **47** is presented displaying the anomeric (F1) region (4 9-5 2 ppm) and the non-anomeric (F2) region (3 5-4 2 ppm) Because of the spectral overlap in the F1 dimension it was not possible to perform a full assignment of the protons

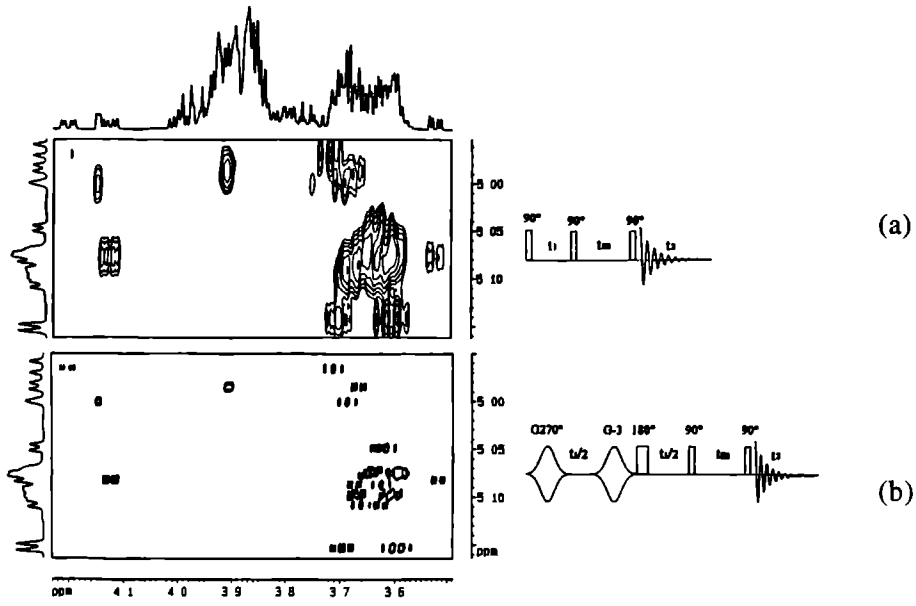


Figure 2.5 *600 MHz 2D ^{1}H-NMR spectra of compound 47 in D_2O, a) classical NOESY, b) semi-soft NOESY decoupled in F1 dimension, mixing time 200 ms*

This overlap could, however, be reduced efficiently using a soft pulse sequence as described by Ernst et al ,[54] see figure 2 5 b This method was used to identify nearly all H-1, H-2, H-3 and H-4 protons, although two glucopyranose units could not be distinguished because the chemical shifts of their anomeric protons were almost identical Therefore an INADEQUATE spectrum was recorded (see Fig 2 6) [55] In this spectrum the F-1 dimension represents the sum of the

of the chemical shifts ($\delta_{H1} + \delta_{H2}$) The two H-1 protons could now be distinguished since their respective δ_{H2} values are different

More experiments involving the use of the DREAM [56] sequence were performed to identify the cyclodextrin protons or to confirm the identification obtained with the other techniques Since the CD protons were located in a very limited band width (0 2 ppm for the anomeric protons and 0 5 ppm for the others) it is a remarkable result that the assignment of 79 of the 100 protons (including the amide proton and excluding the OH-protons) was possible without the use of 3D techniques The chemical shifts of the assigned protons are collected in Table 2 1

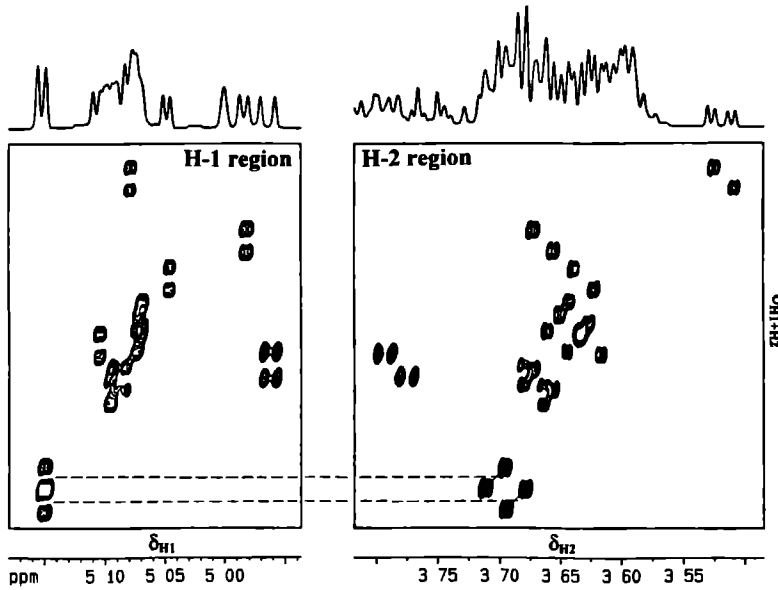
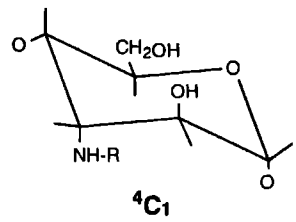


Figure 2.6 *Parts of the 600 MHz semi-soft INADEQUATE spectrum of compound 47 in D_2O*

The most striking results from this Table are the chemical shifts found for the H-3 protons If the values for the units A and H are not considered, the chemical shifts of the H-3 protons of CD I all are between 4 002 and 3 934 ppm, whereas those of CD II are upfield shifted δ-values between 3 860 and 3 666 ppm This suggests that the C_8-spacer is included in the cavity of CD II

Another interesting observation is the difference in coupling constants found for the altrose units A and H For unit A the ^{3}J-coupling constants amounted to J_{12} = 7 2 Hz, J_{23} = 10 7 Hz, J_{34} = 3 8 Hz and $J_H{}^N{}_{H3}$ = 8 8 Hz whereas for unit H the values were J_{12} = 1 7 Hz, J_{23} = 3 2 Hz, J_{34} = 4 3 Hz, J_{45} = 10 4 Hz and $J_H{}^N{}_{H3}$ = 9 0 Hz The coupling constants for the other

glucopyranose-units amounted to J_{12} = approx. 3.6 Hz and J_{23} = 9.7 Hz. The measured coupling constants for units A and H are in agreement with the following conformations: altrose unit A in a $^1C_4$-conformation and unit H in a $^4C_1$-conformation.



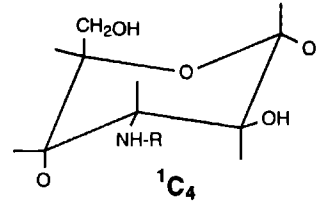
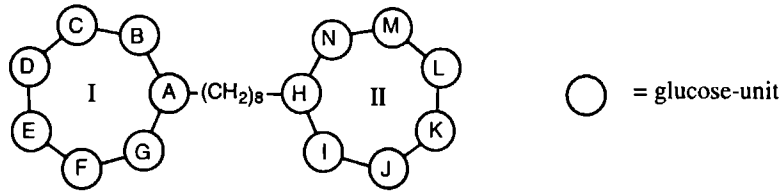


Table 2.1 *Chemical shifts of protons in compound 47*

Pyranoside unit [a]	H-1	H-2	H-3	H-4	H-5	H-6,6'	N-H
A	4.961	3.785	4.203	3.909	4.273	3.812	8.063
B	4.981	3.664	4.002	3.606	3.939	-	
C	5.149	3.687	3.956	3.598	-	-	
D	5.151	3.703	3.937	3.588	-	-	
E	5.076	3.653	3.977	3.621	-	-	
F	5.047	3.630	3.994	3.597	3.898	-	
G	5.069	3.634	3.934	3.710	3.698	-	
H	4.999	4.146	4.552	4.124	3.952	3.791	7.503
I	5.079	3.516	3.666	3.595	-	3.892	
J	5.070	3.643	3.860	3.600	-	-	
K	5.093	3.666	3.861	3.614	3.700	-	
L	5.095	3.672	3.852	3.626	3.701	3.896	
M	5.085	3.674	3.853	3.662	3.706	3.910	
N	5.104	3.625	3.766	3.688	3.759	3.924	

[a] The name giving of the pyranoside units of **47**, seen from their primary sides, is as follows:



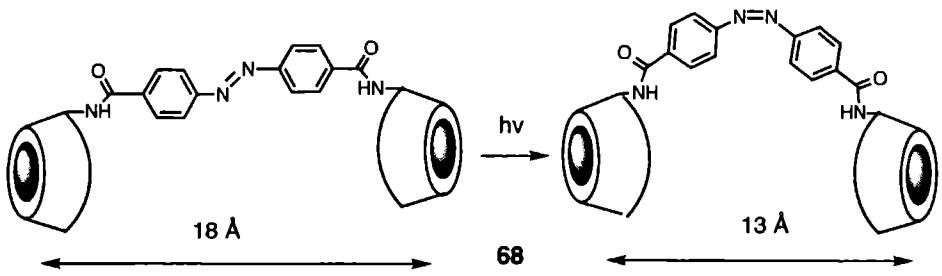
This latter conformation in principle is unfavourable since the large spacer-substituent is located in an axial position The energy required for this conformation is probably gained by the favourable self-inclusion of the spacer in the CD-cavity II An extra stabilising hydrogen bond between the hydroxyl group at C-2 of the altrose unit with the OH group at C-3 of the adjacent glucose unit might also explain the stability of the latter conformation,[57] although no experimental evidence was obtained to support such a hydrogen bond An extra indication that the $^4C_1$-conformation is the result of the self-inclusion of the spacer comes from the coupling constants of the H-1 protons (J_{12} = 7 1 Hz) that are observed if compound **47** is dissolved in DMSO-d6 These values show that in this solvent both units A and H are in the favourable $^1C_4$-conformation

The shifts of the NH-amide-signals in peptides, as a function of the temperature, are used as a measure of the exposure of the amide group to the water phase [58] These so called temperature coefficients were determined for compound **47** in H_2O and amounted to 10 1 and 3 4 ppb per K, for NH_A and NH_H, respectively These numbers indicate that the amide group of CD II is either protected from the solvent or that it forms a strong intramolecular hydrogen bond Either one of the two explanations is in agreement with the self-inclusion of the spacer in the cavity of CD II We, therefore, can conclude that the self-inclusion of the octamethylene linker leads to an induced-fit type of conformational change

2.5.4 Synthesis of an azobenzene-coupled cyclodextrin dimer

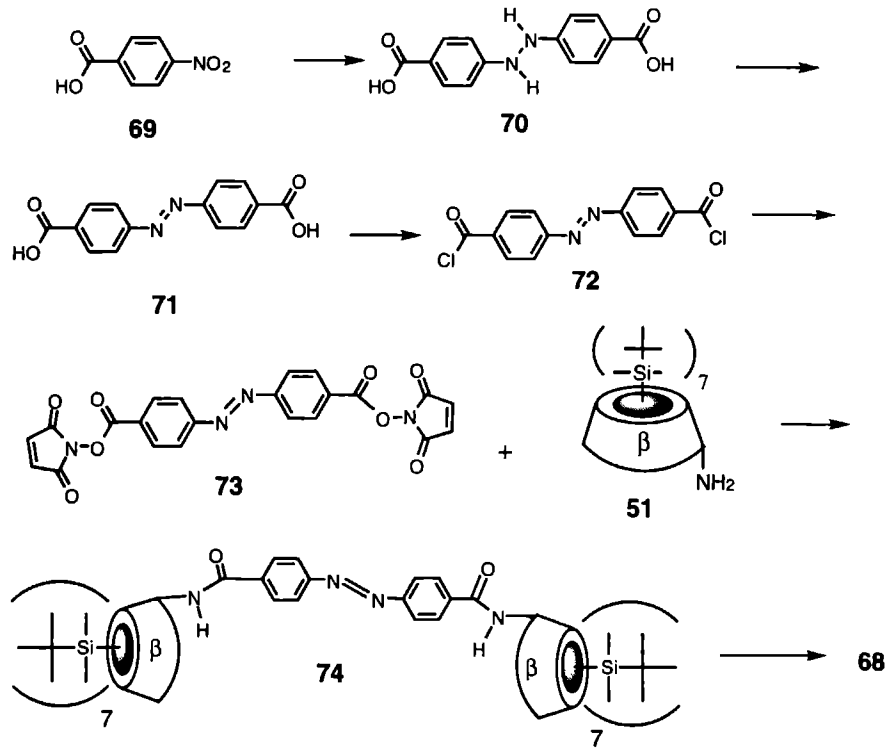
The combination of azobenzene functions with cavity containing molecules is very interesting because it gives the opportunity to "switch on and off" the binding of substrate molecules by using the photo-induced cis/trans isomerisation of the aza bond [59, 60] This cis/trans isomerisation results in a decrease of the distance between the para positions of the two phenyl rings attached to the aza group Cyclodextrins capped with an azobenzene moiety have been prepared for this purpose as was discussed in Chapter 1 [61,62]

A different type of "switch on and off" system is possible on the basis of CD dimers We, therefore, decided to synthesise CD-dimer **68** in which the linking spacer is an azobenzene group We hoped that by photo-irradiating this compound it would be possible to change the



centre to centre distance between the two CD moieties from approx. 13 Å in the cis form to approx. 18 Å in the trans form. This in turn might result in a change in binding affinity for substrate molecules, the trans form binding relatively large substrates and the cis form relatively small substrates.

The synthesis of dimer **68** is shown in Scheme 2.4. First, the azobenzene spacer was synthesised following a procedure described by Tomlinson.[63] Para-nitrobenzoic acid (**69**) was reduced with the help of glucose to the hydrazobenzene **70**. This compound was directly



Scheme 2.4

oxidised with air to the azobenzene **71**. This diacid was converted into the active ester **73** via the diacid chloride **72**. Reaction of the diester with the monoamino-functionalised CD **51** yielded dimer **74** in 52% yield. Desilylation was performed in the same way as described for the alkyl-linked dimers and gave the deprotected dimer **68** in 69% yield. This compound was characterised using FAB-MS and NMR spectroscopy.

The photoisomerisation of the azobenzene moiety of compound **68** was performed by UV-irradiation ($280 < \lambda < 340$ nm) in aqueous solution. The cis content of the photostationary state was approximately 50%. The cis form, which showed a very long lifetime (only 5% cis -> trans isomerisation had occurred after five hours at 25 °C), could be returned to the original trans form by irradiation with visible light in ca. 30 min.. Binding studies were however very difficult to carry out because the azobenzene moiety has a very strong absorption in the UV-vis range (300-500 nm). Titration experiments of dimer **68** with substrates like TNS, methyl orange, methylene blue and porphyrins was not possible, because the relevant absorption bands of the latter molecules always interfered with the wavelength at which the absorption of the azobenzene moiety occurred. Unfortunately compound **68** decomposed slowly during storage, even at low temperatures (-10 °C). The decomposed material could not be identified by NMR. Gel permeation chromatography revealed a species with a molecular weight in the range of β-CD. The compound, therefore, was not subjected to further investigations.

2.6 Experimental

General methods: THF and toluene were distilled from sodium and benzophenone. Ethanol was dried by refluxing for at least 8 h over magnesium (activated by a little iodine) followed by destillation. Pyridine and acetonitrile were dried by refluxing for at least 8 h over CaH_2 (5 $g.l^{-1}$) followed by destillation. DMF was dried by stirring overnight on CaH_2 followed by destillation under reduced pressure (1mm Hg). All dry solvents were kept over molsieves (3 Å). Ethyl acetate was distilled *in vacuo*. All other solvents were used as received. Flash column chromatography of cyclodextrin derivatives was performed on silica gel (particle size < 0.063 mm). Other compounds were purified on silica 60 (Merck). The TLC-plates used were pre-coated silica gel 60 F_{254} on glass plates (Merck). Compounds containing a cyclodextrin unit were detected by spraying with a 10% solution of H_2SO_4 in ethanol followed by heating with a heat gun. Eluents used in chromatography were a mixture (v/v) of ethyl acetate, ethanol and water (A: (100:4:2); B: (100:8:4); C: (100:14:8); D: (100:30:16); F: (100:2:1)) and a mixture (v/v) of n-propanol:ethylacetate:water:ammonia (E: (5:3:3:1)). NMR spectra were recorded on Bruker WH-90, Bruker AC-100 or Bruker AM-400 instruments. Chemical shifts (δ) are reported in ppm downfield from internal $(CH_3)_4Si$. Abbreviations used are s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad. In order to facilitate the

interpretation of the ^{13}C-NMR spectra of the cyclodextrin derivatives, ^{13}C-135-DEPT spectra were recorded which enabled the assignment of the CH_2- and the quarternary carbon atoms FAB-mass spectra were recorded on a VG 7070E instrument or a Finnigan MAT 90 spectrometer The matrix for these measurements was m-nitrobenzylalcohol (NBA) for the silylated cyclodextrin derivatives and glycerol for the desilylated cyclodextrins Melting points were determined on a Reichert Thermopan microscope and are uncorrected IR-spectra were measured on a Perkin Elmer 298 spectrophotometer and the values of the absorption frequencies are given in cm ^{1} Elemental analyses were carried out in the microanalytical department of the University of Nijmegen

2D-NMR experiments 600 MHz ^{1}H-NMR spectra were acquired on a Bruker AMX-600 spectrometer upgraded with a multichannel interface and a cooling unit Sample concentrations were 5 mM and the temperature was set at 298 K For "soft" pulses, typically a 7 ms Gaussian 270° pulse and a 18 5 ms G3 Gaussian Cascade pulse were used for the selective excitation and refocusing of the anomeric proton signals All spectra except RELAYs were acquired in the phase sensitive mode using the TPPI method Full descriptions of the pulse sequences and used methods and techniques will be published by Berthault et al [56]

Heptakis(6-O-*tert*-butyldimethylsilyl)-β-CD (41)

Compound **41** was synthesised according to modified literature methods [41,43] β-cyclodextrin was dried (100 °C, 0 05 mm Hg, 10 h) yielding 33 g of product which was dissolved under violent stirring in 500 ml of dry pyridine At 0 °C 37 28 g (8 5 equiv) of *tert*-butyldimethylsilyl chloride in 100 ml dry pyridine was added in 1 5 h After stirring overnight at room temperature (T_r) the reaction mixture was poored in 1 l of ice/water and stirred for 15 min The white precipitate was filtered (using Celite) and dissolved in 800 ml ethyl acetate, washed twice with 100 ml aqueous 1M HCl, once with 100 ml of a saturated $NaHCO_3$ solution, and once with brine The resulting organic layer was dried ($MgSO_4$) and concentrated *in vacuo* yielding 66 g of crude product Repeated column chromatography (1 4 kg silica, eluent A) resulted in a TLC-pure compound (in our hands, purification by recrystallisation as mentioned in reference 64 did not yield a TLC-pure compound) Yield 44 4 g (79% yield) Mp 287-289 °C (crystals from MeOH/$CHCl_3$, 95/5, v/v) R_f(C)= 0 40 ^{1}H- and ^{13}C-NMR data were in close agreement with reported literature values [43] FAB-MS (m/e) 1957 (M+ Na), 2067 (M + Cs) Anal Calcd for $C_{84}H_{168}O_{35}S_{17}$ $2H_2O$ C, 51 17, H, 8 84 Found C, 51 07, H, 8 85

Mono(2-O-tosyl)heptakis(6-O-*tert*-butyldimethylsilyl)-β-CD (48)

To 15 43 g of dried (80 °C, 0 05 mm Hg, 5h) compound **41** dissolved in 200 ml of dry THF was added 335 mg (1 05 equiv) of a 60% dispersion of NaH in mineral oil The solution was stirred for at least 17 h at room temperature and 1 h at reflux temperature To this solution was added 1 37 g (0 9 equiv) of tosyl chloride After 1 h TLC (C) showed the formation of two major new products The reaction mixture was concentrated *in vacuo*, the product dissolved in ethyl acetate and the solution washed with water/brine (50/50 v/v) and dried ($MgSO_4$) After

removal of the solvent *in vacuo* the resulting 15 5 g of crude product was subjected twice to column chromatography (1 4 kg silica, eluent A) In this way 6 3 g of pure starting material **41** could be recovered Yield 4 56 g (27%, or 46% according to the consumed amount of **41**) Mp 204-206 °C R_f(C)= 0 56 ^{1}H- and ^{13}C-NMR data were in close agreement with reported values [43] FAB-MS (m/e) 2086 (M-) and 2239 5 (M+NBA-1) Anal Calcd for $C_{91}H_{174}O_{37}Si_7S$ C, 52 32, H, 8 40, S, 1 53 Found C, 51 92, H, 8 40, S, 0 99

Mono(2^A,3^A-anhydro)heptakis(6-O-*tert*-butyldimethylsilyl)-β-CD (49)

To a solution of 500 mg of compound **48** in 3 ml of dry ethanol was added 5 ml of sodium ethoxide (55 mg of sodium in 50 ml of dry ethanol) After one night refluxing the reaction mixture was concentrated *in vacuo,* the residue dissolved in ethyl acetate, and the solution washed with water and brine, dried ($MgSO_4$) and concentrated to dryness Further purification was achieved using column chromatography (100 g silica, eluent A, (1l), followed by eluent B) In this way compound **49** was obtained as a white solid Yield 300 mg (65%) Mp 258 °C R_f(C)= 0 38 FAB-MS (m/e) 1938 5 (M+ Na) Anal Calcd for $C_{84}H_{166}O_{34}Si_7 H_2O$ C, 52 15, H, 8 75 Found C, 52 11, H, 8 88

Mono(3-azido-3-deoxy)heptakis(6-O-tert-butyldimethylsilyl)-β-CD. (50)

Compound **48** was dried (2 h, 40 °C, 0 05 mm Hg) to give 1 3 g of product which was converted into the mono-epoxide **49** as described above As soon as the epoxide was the main product in the reaction mixture (according to TLC (C)) 730 mg (20 equiv) of LiN_3 was added After 48 h refluxing the reaction mixture was concentrated *in vacuo* and the product dissolved in ethyl acetate, the solution washed twice with brine/water (1/1, v/v), dried and concentrated The resulting 1 2 g of crude product was purified by column chromatography (150 g silica, eluent A) to give TLC-pure compound **50** resulted as a white solid Yield 880 mg (72%) Mp 236-238 °C (dec) R_f(C)= 0 57 FAB-MS (m/e) 1982 (M + Na) ^{1}H-NMR ($CDCl_3$) 4 95-4 85 (m, 6H, *H*-1), 4 63 (d, J=7 1Hz, 1H, *H*-1), 4 2-3 3 (m, 42H, *H*-2 *H*-3 *H*-4 *H*-5 and *H*-6), 1 3 and 0 9 (2 x m, 9 and 54H, C*H*$_3$-t-Butyl), 0 1 (m, 42H, C*H*$_3$-Si) ^{13}C-NMR ($CDCl_3$)· 105 1 (*C*-1), 102 8 102 5 102 2 101 9 101 3 (*C*-1), 81 5-72 0 (*C*-2 C-3 *C*-4 and *C*-5) 62 1-61 5 (*C*-6), 25 8 (*C*H$_3$-t-Butyl), 18 0 (*C*-t-Butyl), -5 2 (*C*H$_3$-Si) Anal Calcd for $C_{84}H_{167}O_{34}Si_7N_3$ $2H_2O$ C, 50 52, H, 8 68, N, 2 10 Found C, 50 68, H, 8 41, N, 2 06

Mono(3-amino-3-deoxy)heptakis(6-O-tert-butyldimethylsilyl)-β-CD (51)

Method a Via hydrogenation of compound 50
Compound **50** (2 55 g) was dissolved in 100 ml of dry ethanol and hydrogenated using a catalytic amount of Pd/C (10%) and a hydrogen pressure of 3 8 Bar After 40 h, TLC (solvent C) showed 90 % conversion The reaction mixture was filtered over Celite, concentrated and the residue purified by columnchromatography (100 g silica) using eluents (B) and (C) This resulted in the TLC-pure product **51** Yield 1 87 g (75%) Mp 245-255 °C (dec) R_f(C)= 0 1 FAB-MS (m/e) 1934 (M + 1), 1956 (M + Na) ^{1}H-NMR ($CDCl_3$) 4 95-4 75 (m, *H*-1 and *H*DO), 4 2-3 3 (m, 42H, *H*-2 *H*-3 *H*-4 *H*-5 and *H*-6), 1 00-0 75 (m, 63H, C*H*$_3$-t-Butyl), 0 2-0 0 (m, 42H, C*H*$_3$-Si) ^{13}C-NMR ($CDCl_3$) 104 9 (*C*-1), 102 9, 102 6, 102 3, 102 1, 101 8, 101 3 (6 x *C*-1), 82 5-71 9 (*C*-2, *C*-3, *C*-4 and *C*-5) 61 9-61 5 (*C*-6),52 9 (*C*-NH_2), 25 9 ((*C*H$_3$)$_3$-C), 18 2 ((CH$_3$)$_3$-*C*), -5 1 (*C*H$_3$-Si) Anal Calcd for $C_{84}H_{169}O_{34}Si_7N$ $3H_2O$ C, 50 75, H, 8 87, N, 0 70 Found C, 50 70, H, 8 80, N, 0 67

*Method b: Via opening of epoxide **49** with ammonia.*
To a refluxing solution of 2.52 g of dried (80 °C, 0.05 mm Hg, 1h) compound **48** in 20 ml of dry ethanol was added 14.4 ml (1.1 equiv.) of a stock solution of sodium ethoxide in ethanol (106 mg Na in 50 ml ethanol). After the starting material had been converted for more than 90 % (6 h, according to TLC, eluent C), the reaction mixture was cooled to 0 °C and saturated with ammonia gas. This mixture was transferred into an autoclave and heated at 80 °C for 36 h. After removal of the solvent *in vacuo* the crude product was dissolved in dichloromethane. The solution was washed with water and brine, dried ($MgSO_4$) and concentrated to dryness. The crude product (2.5 g) was purified by column chromatography (150 g silica, eluent B followed by eluents C and D). Yield: 1.45 mg (62%). The analytical data of this compound were similar to the data of the product obtained via method a.

Mono(3-amino-3-deoxy)heptakis(6-O-dimethyltexylsilyl)-β-CD (52)

This compound was synthesised following the procedure described for compound **51**, method b (ammonia method). The only difference was the use of 11 equiv. of dimethyltexylsilyl chloride instead of 8.5 equiv. of *tert*-butyldimethylsilyl chloride used for the synthesis of compound **51**. Starting from 3.63 g dried (5h, 50 °C, 0.05 mm Hg) mono(2-O-tosyl) heptakis(6-O-dimethyltexylsilyl)-β-CD, 2.81 g (83% based on the monotosylate) pure product **52** could be obtained. Mp 230 °C (dec). R_f(C)= 0.18. FAB-MS (m/e): 2130.5 (M + 1). Anal. Calcd for $C_{98}H_{197}O_{34}Si_7N$: C, 55.26; H, 9.32; N, 0.66. Found : C, 55.05; H, 9.81; N, 0.80.

Succinic acid bis(4-nitrophenyl)ester (53)

Succinic acid (2.04 g), *p*-nitrophenol (6.01 g) and a catalytic amount of 4-dimethylaminopyridine (DMAP) were dissolved in 50 ml of dichloromethane. At 0 °C, a solution of 7.47 g of *N,N'*-dicyclohexylcarbodiimide in 25 ml of dichloromethane was added. After 25 h at room temperature the reaction mixture was filtered and evaporated to dryness. Purification of the crude product by column chromatography (silica 60, 150g, CH_2Cl_2) resulted in decomposition of the compound. The product could however be purified by crystallisation from chloroform. Yield 2.11 g (34 %). Mp 184 °C. CI-MS (m/e): 361.2 (M + 1). ^{1}H-NMR (DMSO-d6) : 8.30 (d, J=9.0 Hz, 4H, *Ar-H*), 7.39 (d, J=9.0 Hz, 4H, *Ar-H*), 3.06 (s,4H,CH_2). Anal. Calcd for $C_{16}H_{12}O_8N_2$: C, 53.34; H, 3.36; N, 7.78. Found : C, 53.26; H, 3.34; N, 7.72.

Sebacic acid bis(4-nitrophenyl)ester (54)

Sebacic acid (4.85 g), *p*-nitrophenol (10.04 g) and a catalytic amount of DMAP were dissolved in 50 ml of dichloromethane. At 0 °C a solution of 11.60 g of *N,N'*-dicyclohexylcarbodiimide in 25 ml dichloromethane was added. After the reaction had been completed (10 h at room temperature) the reaction mixture was filtered and concentrated *in vacuo*. The resulting dry product was purified by repeated crystallisations from acetonitrile. Yield 6.39 g (60 %). Mp 107 °C. CI-MS (m/e): 306 (M-*p*-nitrophenol). ^{1}H-NMR (DMSO-d6) : 8.13 (d, J=9.0 Hz, 4H, Ar-*H*), 7.13 (d, J=9.0 Hz, 4H, Ar-*H*), 2.5 (t,4H,CH_2), 1.8-1.1 (m, 12H, CH_2). Anal. Calcd for $C_{22}H_{24}O_8N_2$: C, 59.46; H, 5.44; N, 6.30. Found : C, 59.82; H, 5.41; N, 6.58.

N,N'-Bis[mono(3-deoxy)heptakis(6-O-dimethyltexylsilyl)-β-CD]-butan-1,4-diamide (55)

Monoamino-functionalised cyclodextrin **52** was thoroughly dried (40 °C, 4 h, 0.05 mm Hg) and 1.82 g of the dry compound was dissolved in 25 ml of THF. After addition of 0.15 g of compound **53** the reaction mixture was refluxed until the reaction was completed according to TLC (24 h). The solvent was evaporated, and the residue was dissolved in dichloromethane. This solution was washed with a saturated aqueous solution of $NaHCO_3$ (twice) and with brine (twice), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude product was subjected to column chromatography (150 g silica, eluent B). Yield: 1.35 g (76%). Mp 260 °C (dec). R_f(D)= 0.54. IR: 1680-1610, 1570-1550 (amide I and II). ^{1}H-NMR ($CDCl_3$) : 6.56-5.82 (O*H*), 5.57-4.80 (3 x m, *H*-1 and O*H*), 3.92-3.45 (m, ca. 86H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6), 3.28 (t, 2H, *H*-4), 2.96-2.69 (m, 4H, CH_2-spacer), 2.17-1.98, 1.67-1.56 (s, 84H, CH_3-Si), 1.28-1.24 (m, 3H), 0.98-082 (m, 168H, CH_3-C), 0.12-0.07 (s, 84H, CH_3-Si). ^{13}C-NMR ($CDCl_3$): 172.22 (*C*O), 105.18-101.91 (*C*-1), 81.66, 81.46 and 74.97-67.11 (*C*-2, *C*-3, *C*-4 and *C*-5), 63.17-61.01 (*C*-6), 52.47 (*C*-NH), 34.18 (*C*H-texyl), 32.73 (*C*H_2-spacer), 25.17, 25.05, 23.04, 20.98, 18.47 (*C*-texyl), -3.02, -3.30 and -3.44 (*C*H_3-Si). Anal. Calcd for $C_{200}H_{396}O_{76}Si_{14}N_2.H_2O$: C, 55.12; H, 9.20; N, 0.64. Found : C, 55.12; H, 9.92; N, 0.73.

N,N'-Bis[mono(3-deoxy)heptakis(6-O-*tert*-butyldimethylsilyl)-β-CD]-decan-1,10-diamide (56)

Monoamino-functionalised β-cyclodextrin **51** was dried thoroughly (40 °C, 4 h, 0.05 mm Hg) and 1.65 g of the dry compound was dissolved in 25 ml of THF. After addition of 0.18 g of compound **54** the reaction mixture was refluxed. After 24 h the reaction was not completed. Addition of extra **51** and prolongation of the reaction time did not result in a complete reaction. After 2 days the reaction mixture was concentrated and the residue dissolved in dichloromethane. The solution was washed with an aqueous saturated solution of $NaHCO_3$ (twice) and with brine (twice), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude product was subjected two times to column chromatography (150 g silica, eluent B; 150 g silica, eluent A). Yield: 1.63 g (57%). Mp 265-270 °C (dec). R_f(C)= 0.28. IR: 1680-1610, 1570-1550 (amide I and II). FAB-MS (m/e): 4028.2 (M - 3). Anal. Calcd for $C_{178}H_{352}O_{70}Si_{14}N_2.H_2O$: C, 52.76; H, 8.81; N, 0.69. Found : C, 52.05; H, 8.72; N, 0.79.

N,N'-Bis[mono(3-deoxy)-β-CD]-butan-1,4-diamide (46)

To a solution of 0.77 g of dimer **55** (dried at 40 °C, 2 h, 0.05 mm Hg) in 25 ml of THF was added 3.20 ml of a 1.0 M solution of TBAF in THF. After 18 h refluxing, 25 ml of water was added and the reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in a minimum amount of water and precipitated in 100 ml of ethanol (analytical grade). The precipitate was collected by centrifugation. The precipitation was repeated two times to remove all tetrabutylammonium salts. Yield: 0.39 g (95%). Mp 265-268 °C. R_f(E)= 0.1. IR: 1680-1610 and 1560-1550 (amide I and II). FAB-MS (m/e, glycerol): 2349.9 (M + 1). ^{1}H-NMR (D_2O) : 5.09 (d, 1H, *H*-1), 5.03-4.96 (m, 12H, *H*-1), 4.90 (d, 1H, *H*-1), 4.21-4.15 (m, 4H), 3.96-3.75 and 3.66-3.52 (2 x m, ca. 84H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6), 2.57 (CH_2-spacer). ^{13}C-NMR (D_2O): 176.12, 105.06-102.41, 82.32-81.21, 74.41-71.11, 61.58, 60.97, 59.38, 52.20, 32.28. Anal. Calcd for $C_{88}H_{144}N_2O_{70}.15\ H_2O$: C, 40.34; H, 6.69; N, 1.07. Found : C, 39.92; H, 6.52; N, 1.07.

N,N'-Bis[mono(3-deoxy)-β-CD]-decan-1,10-diamide (47)

To a solution of 0 59 g of dimer **56** (dried at 40 °C, 3 h, 0 05 mm Hg) in 25 ml of THF was added 2 70 ml of a 1 0 M solution of TBAF in THF After 36 h refluxing, 25 ml of water was added and the reaction mixture was concentrated *in vacuo* The resulting residue was dissolved in a minimum amount of water and precipitated in 100 ml of ethanol The solid material was collected by centrifugation This precipitation procedure was repeated three times to remove all tetrabutylammonium salts Yield 0 31 g (88%) Mp 246-248 °C R_f(E)= 0 1 IR 1670-1610 and 1560-1550 (amide I and II) FAB-MS (-m/e, glycerol) 2430 5 (M - 2) ^{1}H-NMR (D_2O) 5 10 and 5 02-4 90 (d and m, 14H, *H*-1), 4 09 (m, 4H), 3 94-3 71 and 3 66-3 35 (2 x m, ca 84H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6), 2 38-2 13 (2 x m, 4H, C*H*$_2$-spacer), 1 70-1 55 (2 x m, 4H, C*H*$_2$-spacer), 1 50-1 32 (2 x m, 4H, C*H*$_2$-spacer), 1 25-1 13 (2 x m, 4H, C*H*$_2$-spacer) ^{13}C-NMR (D_2O) 177 84 and 176 98 (*C*O), 105 02-102 30 (*C*-1), 82 41-80 05 and 77 86-69 06 (*C*-2, *C*-3, *C*-4 and *C*-5), 61 90-60 71 (*C*-6), 52 45 and 52 13 (*C*-NH), 37 62, 36 87, 30 28, 30 16, 29 12, 28 77, 26 95 and 26 32 (8 x C*H*$_2$-spacer) Anal Calcd for $C_{94}H_{156}N_2O_{70}$ 7 H_2O C, 44 10, H, 6 69, N, 1 09 Found C, 44 01, H, 6 91, N, 1 17

Hexakis(6-O-*tert*-butyldimethylsilyl)-α-CD (58)

To a solution of 19 0 g of dried (100 °C, 0 05 mm Hg, 9 h) α-cyclodextrin in 350 ml of THF was added at 0 °C over a period of 1h 22 6 g (7 7 equiv) of *tert*-butyldimethylsilyl chloride in 50 ml of dry pyridine After stirring for 24 h at room temperature the reaction mixture was pored in 1 l of ice/water and stirred for 15 min The white precipitate was filtered over Celite and dissolved in dichloromethane The solution was washed twice with HCl (1 M), once with 100 ml of a saturated $NaHCO_3$ solution and once with brine The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to yield 40 g of crude product Repeated column chromatography (1 6 kg of silica, eluent A) resulted in a TLC-pure compound Yield 22 6 g (69% yield) Mp 331 °C (dec), (lit [42] 323-326 °C, dec), R_f(C)= 0 4 ^{1}H-NMR ($CDCl_3$) 4 88 (d, 6H, *H*-1), 4 01 (t, 6H), 3 91 (dd, 6H), 3 84 (d, 6H), 3 75 (d, 6H), 3 64 (dd, 6H), 3 59 (t, 6H), 0 89 (s, 54H, C*H*$_3$-C), 0 03 (s, 36H, C*H*$_3$-Si) ^{13}C-NMR ($CDCl_3$) 101 39 (*C*-1), 81 40, 74 45, 73 04 and 72 19 (*C*-2, *C*-3, *C*-4 and*C*-5), 61 95 (*C*-6), 25 97 (*C*H$_3$-C), 18 42 (CH$_3$-*C*), -5 19 (*C*H$_3$-Si) FAB-MS (m/e) 1681 (M+ 1) Anal Calcd for $C_{72}H_{144}O_{30}Si_6$ C, 52 15, H, 8 75 Found C, 52 39, H, 8 53

Mono(2-O-tosyl)hexakis(6-O-*tert*-butyldimethylsilyl)-α-CD (59)

To a solution of 2 87 g of dry compound **58** (dried at 80 °C, 0 05 mm Hg, 6 h) in 150 ml of dry THF was added 103 mg (1 5 equiv) of a 60% dispersion of NaH in mineral oil The solution was stirred for at least 17 h at room temperature The reaction mixture was heated to reflux temperature and 495 mg (1 5 equiv) of tosyl chloride was added After 2 h refluxing, the reaction mixture was concentrated *in vacuo*, the residue dissolved in ethyl acetate and the resulting solution washed with water/brine (50/50 v/v) and twice with brine, dried ($MgSO_4$) and evaporated The crude product (3 0 g) was subjected twice to column chromatography(first run 800 g silica, eluent A, second run 800g silica, eluent F) In this way 600 mg (21%) of pure starting material **58** could be recovered Yield 800 mg (25%) Mp 218 5 °C (dec) ^{1}H-NMR ($CDCl_3$ CD_3OD, 10 1, v v) 7 89 (d, 2H, Ar-*H*), 7 31 (d, 2H, Ar-*H*), 5 11, 4 91, 4 86, 4 81, 4 76 (5xd, total 6H, *H*-1), 4 13-3 90 and 3 76-3 49 (4 x m, 35 H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6),

3 32 (dd, 1H), 2 43 (s, 3H, Ar-C*H*$_3$), 0 88 (s, 54H, C-C*H*$_3$), 0 04 (s, 36H, Si-C*H*$_3$) ^{13}C-NMR ($CDCl_3$ CD_3OD, 10 1, v v) 145 42 and 132 03 (*C*-Ar), 129 51, 129 05 (*C*H-Ar), 102 95-101 36 and 99 32 (*C*-1), 81 62-80 03, 73 46-71 91, 69 32 (*C*-2,*C*-3,*C*-4,*C*-5), 62 11-61 94 (*C*-6), 25 96 (CH_3-Tos), 21 71 (CH_3-C), 18 43 and 18 29 ((CH_3)$_3$-*C*), -5 07 and -5 20 (CH_3-Si) Anal Calcd for $C_{79}H_{150}O_{32}Si_6S$ C, 52 35, H, 8 34, S, 1 77 Found C, 52 34, H,8 30, S, 1 63

Mono(3-amino-3-deoxy)hexakis(6-O-tert-butyldimethylsilyl)-α-CD (61)

To a refluxing solution of 700 mg of dried (100 °C, 0 05 mm Hg, 1h) compound **59** in 60 ml of dry ethanol was added 6 0 ml of a stock solution of sodium ethoxide in ethanol(81 mg Na in 50 ml ethanol) After more than 90 % of compound **60** had been formed (according to TLC, eluent C), the reaction mixture was cooled to 0 °C and ammonia gas was bubbled though the solution until saturation The resulting mixture was heated in an autoclave at 80 °C for 50 h Removal of the solvent *in vacuo* resulted in a crude product which was purified by column chromatography (50 g silica, eluent B) Yield 405 mg (63%) (dec) R_f(D)= 0 42 FAB-MS (m/e) 1658 (M + 1), 1789 (M + Cs) ^{1}H-NMR ($CDCl_3$ CD_3OD, 2 1, v/v) 4 77 and 4 73 (2 x br s, 5H, *H*-1), 4 52 (d, J=6 Hz,1H, H^A-1), 4 05-3 23 and 3 03 (4 x m, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6 and CD_3OH), 0 75 (s, 54H, (C*H*$_3$)$_3$-C), -0 05 (m, 36H, C*H*$_3$-Si) ^{13}C-NMR ($CDCl_3$ CD_3OD, 2 1, v/v) 104 98, 102 70, 102 50, 101 89 and 100 85 (*C*-1), 81 05-71 61 (*C*-2, *C*-3, *C*-4 and *C*-5) 62 37-61 33 (*C*-6), 52 09 (*C*-NH_2), 25 65-25 54 (CH_3-C), 18 02-17 87 (*C*H$_3$)$_3$-C), -5 44- -5 84 (CH_3-Si) Anal Calcd for $C_{72}H_{145}O_{29}Si_6N$ $2H_2O$ C, 51 07, H, 8 87, N, 0 83 Found C, 51 36, H, 8 74, N, 0 80

Coupling product of β-cyclodextrin derivative 51 with compound 53 (62)

To a solution of 334 mg of dried (80 °C, 2 h, 0 05 mm Hg) compound **51** in 30 ml of THF was added 500 mg (8 equiv) of compound **53** The reaction mixture was heated at 40 °C for 24 h and subsequently concentrated *in vacuo* The crude product was immediately subjected to column chromatography (10 g silica, eluent A) To prevent decomposition during the purification procedure the column should not be too long and the elution rate not be too small Yield 188 mg (51%) R_f(C)= 0 53 FAB-MS (-m/e) 2154 (M -), 2014 (M - p-nitrophenol) Because of its instability, the product was directly converted into compound **64** without further analysis

Coupling product of β-cyclodextrin derivative 51 with compound 54 (63)

To a solution of 280 mg of dried (100 °C, 1 h, 0 05 mm Hg) monoamino-functionalised cyclodextrin **51** in 25 ml of THF was added 760 mg (12 equiv) of compound **54** The reaction mixture was heated at 40 °C for 12 h After this period, approximately 80% of a new product had been formed according to TLC (eluent C) The reaction mixture was then concentrated *in vacuo* The crude product was immediately subjected to column chromatography (75 g silica, eluent A) The decomposition of **63** during purification was less (but not negligible) than observed for compound **62** Yield 205 mg (63%) R_f(C)= 0 47 FAB-MS (m/e) 2239 5 (M +), 2261 (M + Na) ^{1}H-NMR ($CDCl_3$, 90 MHz) 8 70 and 7 35 (2 x d, 4H, Ar-*H*), 4 90 (br signal, 7H, *H*-1), 4 30-3 50 (br m, ca 42H), 2 70, 2 50, 1,80, 1 40 (4 x br m, approx 16H, C*H*$_2$-spacer), 0 9 (br s, ca 63H, C*H*$_3$-C), 0 0 (br s, ca 42H, C*H*$_3$-Si)

N-[Mono(3-deoxy)heptakis(6-O-*tert*-butyldimethylsilyl)-β-CD]-N'-[mono(3-deoxy)hexakis(6-O-*tert*-butyldimethylsilyl)-α-CD]-butan-1,4-diamide (64)

To a solution of 188 mg of compound **62** in 10 ml of THF was added 150 mg of compound **61**. The reaction mixture was refluxed until the reaction was completed according to TLC (eluent C, 20 h). After concentration *in vacuo* the residue was directly subjected to column chromatography (25 g silica, eluent A). Yield: 195 mg (61%). FAB-MS (m/e): 3673 (M +). ^{1}H-NMR ($CDCl_3$,90 MHz) : 4.80 (br.signal, *H*-1), 4.20-3.40 (br. m), 2.5 (br. signal, CH_2-spacer), 0.8 (br. s, CH_3-C), 0.0 (br. s, CH_3-Si).

N-[Mono(3-deoxy)heptakis(6-O-*tert*-butyldimethylsilyl)-β-CD]-N'-[mono(3-deoxy)hexakis(6-O-*tert*-butyldimethylsilyl)-α-CD]-decan-1,10-diamide (65)

This compound was synthesised from 135 mg of compound **63** as described for **64**. The crude product was purified by column chromatography (40 g silica, eluent A). Yield: 180 mg (80%). FAB-MS (-m/e): 3755 (M+).

N-[Mono(3-deoxy)-β-CD]-N'-[mono(3-deoxy)-α-CD]-butan-1,4-diamide (66)

To a solution of 140 mg of compound **64** in 10 ml of THF was added 0.57 ml of a 1.0 M stock solution of TBAF in THF (15 equiv.). The mixture was refluxed (24 h) until the reaction was completed according to TLC (eluent E). The reaction mixture was concentrated *in vacuo* and the residue dissolved in a minimum amount of water. This solution was poured into acetone yielding a white precipitate. Repeating this procedure twice afforded pure compound **66**. Yield: 42 mg (50%). Mp >280 °C (dec). R_f(E)= 0.1. IR: 1680-1610, 1560-1520 (amide I and II). FAB-MS (m/e): 2189 (M + 1). ^{1}H-NMR (D_2O) : 5.06 (m, 3H, *H*-1), 5.00 (m, 6H, *H*-1), 4.95 (m, 2H, *H*-1), 4.89 (m, 2H, *H*-1), 4.17-4.13 (m, 4H), 3.93-3.76 and 3.64-3.52 (2 x m, ca. 74H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6), 2.56 (br. s, 4H, CH_2-spacer). ^{13}C-NMR (D_2O): 176.07 (*C*-carbonyl), 105.48 and 105.00 (*C*-1^A and *C*-1$^{A'}$), 103.16-101.62 (*C*-1), 82.79-81.21, 79.75, 77.28, 74.37-72.43, 71.36 and 71.06 (*C*-2, *C*-3, *C*-4 and *C*-5), 62.01-60.91 (*C*-6), 52.19 and 51.98 (*C*-3^A and *C*-3$^{A'}$), 32.25 (CH_2-spacer). Anal. Calcd for $C_{82}H_{134}N_2O_{65}.5H_2O$: C, 43.24; H, 6.37; N, 1.23. Found : C, 43.08; H, 6.30; N, 1.30.

N-[Mono(3-deoxy)-β-CD]-N'-[mono(3-deoxy)-α-CD]-decan-1,10-diamide (67)

To a solution of 140 mg of compound **65** in 10 ml of THF was added 0.56 ml of a 1.0 M stock solution of TBAF in THF (15 equiv.). The mixture was refluxed (15 h) until the reaction was completed according to TLC (eluent E). The reaction mixture was concentrated *in vacuo* and the product dissolved in a minimum amount of water. The solution was poured into ethanol (analytical grade) yielding a white precipitate. Repeating this precipitation afforded compound **66**, which was still contaminated with tetrabutylammonium salts. The latter salts could be removed by running the compound, dissolved in water, over a cation exchange column in the NH_4^+-form. Yield: 60 mg (71%). Mp >310 °C (dec). R_f(E)= 0.1. IR: 1670-1600 and 1560-1520 (amide I and II). FAB-MS (-m/e): 2271 (M - 1). ^{1}H-NMR (DMSO-d6) : 4.90-4.57 (5 x m, 13H, *H*-1), 4.03 (dd, 2H), 3.90 (m, 2H), 3.76-3.25 (2 x m, ca. 74H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6), 2.06 (br. t, 4H, CH_2-CO) 1.48 (br. s, 4H, CH_2-spacer), 1.24 (br.s, 8H, CH_2-spacer). ^{13}C-NMR (DMSO-d6): 172.21 and 172.03 (2 x *C*-carbonyl), 104.72 and 104.35 (*C*-1^A and *C*-1$^{A'}$), 102.49-101.15 (*C*-1), 82.92, 81.96-80.53, 79.66, 79.47, 76.59, 73.54-

71 72, 70 58 (*C*-2, *C*-3, *C*-4 and *C*-5), 59 92-59 45 (*C*-6), 50 20 and 49 63 (2 x CH_2-CO-spacer), 35 65, 28 87 and 25 27 (CH_2-spacer) Anal Calcd for $C_{88}H_{146}N_2O_{65}$ $4H_2O$ C, 45 09, H, 6 62, N, 1 20 Found C, 45 12, H, 6 49, N, 1 24

Azobenzene-4,4'-dicarboxyl chloride (72)

Compound **72** was synthesised from *p*-nitrobenzoic acid as described by Tomlinson [63] Mp 164-165 °C (lit[63] 164) ^{1}H-NMR ($CDCl_3$,90 MHz) 8 25 and 8 05 (2 x d,2 x 4H, *H*-Ar)

Azobenzene-4,4'-dicarboxylic acid, bis(*N*-hydroxysuccinimide)ester (73)

To a solution of 200 mg of compound **72** in 25 ml of dry dichloromethane was added 3 equiv (307 mg) of freshly prepared potassium *N*-hydroxysuccinimide [65] After stirring for 2 h the reaction mixture was concentrated *in vacuo* and the residue dissolved in 300 ml of dichloromethane The resulting solution was washed with a saturated aqueous solution of $NaHCO_3$ and with brine (twice), dried ($MgSO_4$), filtered and concentrated *in vacuo* The resulting red powder was pure according to TLC (eluent 10% MeOH in $CHCl_3$, R_f-value 0 68) Yield 270 mg (87%) Mp 292-294 °C ^{1}H-NMR ($CDCl_3$, 90 MHz) 8 35 (d, 4H, *H*-Ar), 8 07 (d, 4H, *H*-Ar), 2 98 (s, 8H, CH_2)

Silylated cyclodextrin dimer with azobenzene spacer (74)

Compound **51** was dried (100 °C, 0 05 mm Hg, 1 h) (550 mg) and dissolved in 15 ml of THF At reflux temperature, 57 mg (0 95 equiv) of compound **72** was added After 40 h refluxing, the reaction mixture was concentrated *in vacuo* and the residue directly subjected to column chromatography (50 g silica, eluent F, followed by eluent A) which yielded pure dimer **74** Yield 290 mg (52%) R_f(C)=0 3 The NMR signals of this compound were extremely broad This might be due to the presence of various conformations in solution ^{1}H-NMR ($CDCl_3$ CD_3OD, 9 1, v v) 8 10-7 55 (4 x br s,8H,Ar-*H*), 4 98-4 40 (br m, 14H,*H*-1), 4 00-3 25 (br m,*H*-CD-unit + CD_3O*H*), 0 85-0 70 (2 x br s,ca 126H,CH_3-tert-butyl), 0 0-(-0 09) (3 x br s,ca 84H,CH_3-Si) Raising the temperature to 50°C to achieve a better spectrum resulted in 10% decomposition of the compound (as indicated by TLC) Anal Calcd for $C_{182}H_{342}N_4O_{70}Si_{14}$ C, 53 32, H, 8 42, N, 1 37 Found C, 52 46, H, 8 27, N, 1 46

Cyclodextrin dimer with azobenzene spacer (68)

To a solution compound **74** (250 mg) in 15 ml of THF was added 1 1 ml of a 1 M stock solution of TBAF in THF This mixture was stirred overnight at 40 °C and subsequently concentrated *in vacuo* The residue was dissolved in a minimum amount of water, and the product precipitated as an orange solid by addition of ethanol and pentane Yield 105 mg (69%) FAB-MS (-m/e) 2501 6 (M - 1) and a small signal at 1386 (M - β-CD + 19) which indicated some decomposition ^{1}H NMR (DMSO-d6) 8 07 (d,4H,Ar-*H*), 7 97 (d,4H,Ar-*H*), 5 92-5 50 (signals from OH-groups) 5 02-4 70 (5 x m, *H*-1 + signals from OH-groups), 4 50-4 35 (m, signals from OH groups), 3 99 (br m, 2H) 3 82-3 29 (2 x m, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6) ^{13}C-NMR (DMSO-d6) 165 39 (*C*-carbonyl), 153 18 (*Ar C*--N=N), 137 05 (*Ar-C*--C=O), 128 79 and 122 62 (*Ar-C* H), 104 19, 102 06, 101 80 and 101 55 (*C*-1), 81 89, 81 43, 81 03, 80 59, 79 43, 77 46, 73 20-70 86 (*C*-2, *C*-3, *C*-4 and *C*-5), 60 26-59 53 and 57 53 (*C*-6), 51 44 (C-3^A)

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CHAPTER 3

Synthesis of Novel Cyclodextrin Dimers and their Application in the Catalytic Epoxidation of Nerol

3.1 Introduction

Cyclodextrins (CDs) are interesting naturally occurring molecules which can play an important role in catalytic processes in aqueous media because they possess a non-polar cavity. This cavity, for example, can be used to bind a substrate close to a catalytic metal centre by hydrophobic interactions. This makes CDs attractive building blocks for the construction of enzyme models.[1] Cyclodextrin dimers are even more attractive in this respect because these molecules are able, at least in principle, to bind a substrate in a fixed geometry with respect to a catalytic metal centre by making use of two cavities instead of one. For example, Breslow et al. have achieved a 10^5-fold rate enhancement of an ester hydrolysis, as compared to the uncatalysed reaction, with the help of a CD-dimer.[2] In this chapter we describe the synthesis of two novel CD dimers which were designed to serve as components in a Cytochrome P-450 mimic. One of these dimers was tested in a catalytic system for the epoxidation of nerol with molecular oxygen.

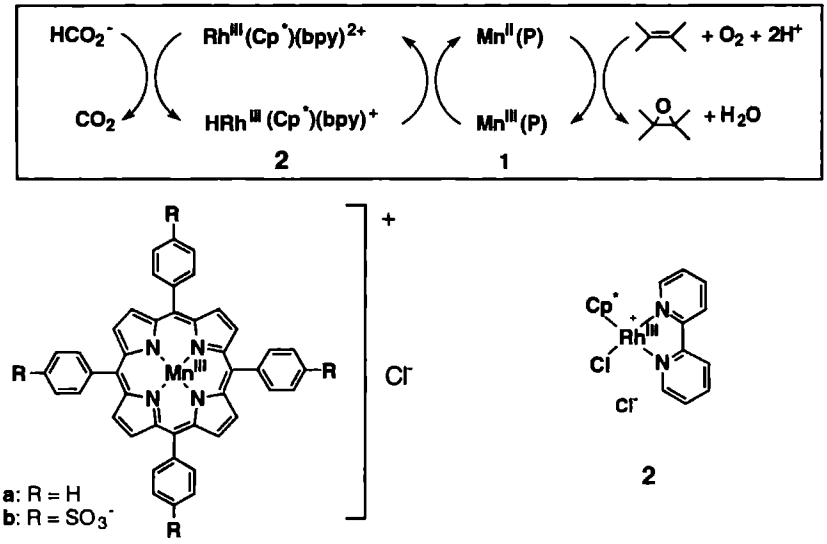
3.1.1 Cytochrome P-450

Cytochrome P-450 dependent monooxygenases are membrane-bound enzymes which catalyse a great variety of oxidation reactions, e.g. the hydroxylation of alkanes and the epoxidation of alkenes, at room temperature with molecular oxygen as the oxidant and NAD(P)H as the electron donor (equation 1).[3,4] Catalysts that allow reactions to proceed under mild conditions

$$R + NAD(P)H + H^+ + O_2 \longrightarrow RO + H_2O + NAD(P)^+ \qquad (1)$$

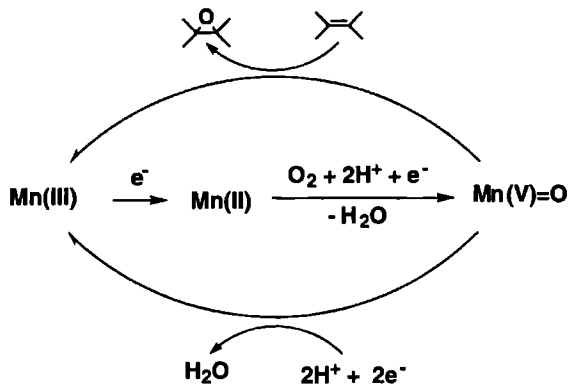
with a readily available reagent, as molecular oxygen is, are of great potential industrial use. Many research groups, therefore, have been working on synthetic model systems mimicking

the enzymatic reactions of Cytochrome P-450. In the natural system the active catalyst is an iron protoporphyrin IX group which contains the thiolate function of a cysteine residue as an axial ligand as was concluded from the crystal structure of the enzyme.[5] Most of the enzyme mimics feature a metalloporphyrin (usually a manganese porphyrin) as catalyst and *N*-methyl imidazole or pyridine as the axial ligand. This porphyrin is reduced by a co-catalyst like sodium borohydride, ascorbate, zinc, Pt/H_2 or *N*-methyldihydronicotinamide.[3] One of the systems that has been investigated by our group is based on the redox cycle shown in Scheme 3.1.



Scheme 3.1

In this redox cycle an alkene is epoxidised by molecular oxygen with manganese porphyrin **1a** as catalyst. This porphyrin is efficiently reduced by the action of the rhodium complex **2** and sodium formate. This combination was used as the reductant in a two-phase system [6] and in a system in which both the rhodium compound and the porphyrin were incorporated in vesicles.[7] Studies on the mechanism of the reductive activation of molecular oxygen catalysed by metalloporphyrins have shown that the crucial steps are the transfer of two electrons from the cofactor reductant to the (Mn(III))-porphyrin molecule. The first electron is used to generate a Mn(II) porphyrin. This reduced porphyrin reacts with another electron, two protons and molecular oxygen to give what is formally a Mn(V)-oxo species and a water molecule, as is depicted in Scheme 3.2. The Mn(V)-oxo species can react with an alkene to give the corresponding epoxide. Alternatively, further supply of electrons to the Mn(V)-oxo species will lead, via a so called "non-productive route", to the formation of water.[8,9]



Scheme 3.2

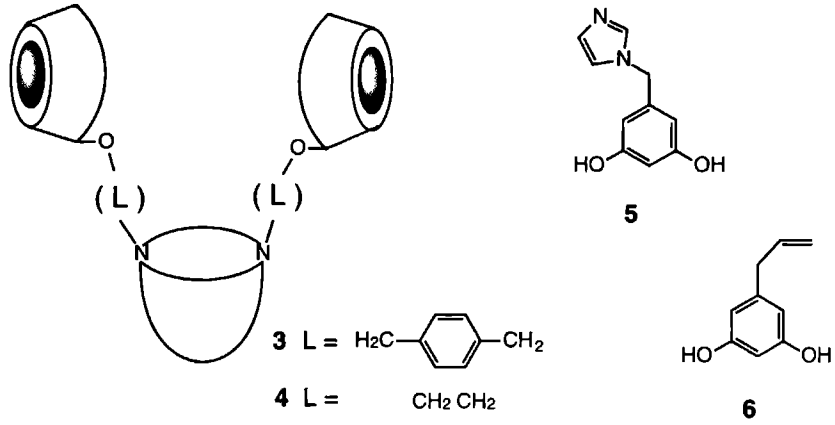
In systems studied by Tabushi et al [10] it was shown that this non-productive pathway can be very competitive and be highly dependent on the reducing agent used For example, in a homogeneous system of manganese tetrasulphonatoporphyrin **1b** in ethanol/water (1/1, v/v) the observed ratio of rate constants (epoxide formation)/(non productive consumption) was 1 x 10 [7] when the combination colloidal Pt and H_2 was used as the electron source, whereas this ratio was 0 03 when *N*-methyldihydronicotinamide (MeNAH), which is a two electron donor, was applied in the presence of flavin mononucleotide [10] This was explained by an overflow of electrons to the Mn(V)-oxo species in the case of the Pt/H_2 system which could not occur in the case of MeNAH

A process that limits the efficiency of porphyrins as catalyst in epoxidation reactions, is the formation of unreactive μ-oxo dimers which are formed by reaction of a Mn(V)-oxo complex with a Mn(III)-porphyrin This dimer formation is believed to be the first step in the process that leads to the destruction of the porphyrin and therefore should be suppressed [11] This can be realised by using the picket fence porphyrins described by Collman et al ,[12] which are porphyrins modified with bulky side groups that sterically hinder the formation of μ-oxo dimers The same protecting effect can be achieved by incorporating the porphyrins in vesicles [7 13] or by encapsulating them in CDs [14] In the Cytochrome P-450 enzyme μ-oxo dimer formation is prohibited by encapsulation of the porphyrin in a protein coat

In order to develop an efficient catalytic system based on the Cytochrome P-450 catalytic cycle we followed two strategies which will be described in the following sections

3.1.2 Bis cyclodextrin modified basket molecules as frameworks in a Cytochrome P-450 mimic

The first strategy makes use of a CD dimer that has a third binding place in the linker between the two CD rings. With this dimer as framework some of the required components for the catalytic cycle (porphyrin, alkene or ligand) can be fixed in a well-defined geometry. The two CD dimers that were designed are shown underneath.



Both compounds consist of two CD-moieties that are connected via their secondary side by a basket-shaped receptor molecule. The latter receptor is derived from diphenylglycoluril. Molecules of this type have been extensively investigated in our group and are known to bind dihydroxybenzene guest molecules.[15] In the context of the design of a Cytochrome P-450 mimic, the basket-shaped linker can be used to immobilise olefins, like substrate **6**, [16] or axial ligands like **5**.

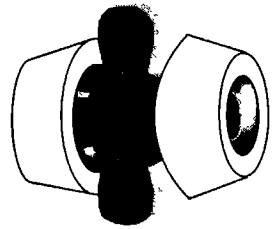


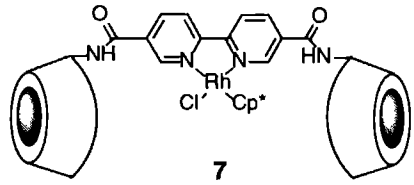
Figure 3.1 *Schematic representation of a supramolecular complex between porphyrin* ***1b*** *and two β-cyclodextrin molecules*

Recent studies in the literature [17] and our own investigations described in Chapter 4 have shown that two β-CDs can encapsulate the metal-free derivative of porphyrin **1b** (Figure 3.1).

From CPK models it appeared that such a complex can also be formed between a mangansese(III)porphyrin and our target molecules **3** and **4**. The encapsulation of the porphyrin will prevent the formation of μ-oxo dimers and therefore protect it from degradation. Also, a good substrate selectivity can be expected if substrates (like **6**) are bound in the basket shaped linker. Alternatively, this extra recognition site can be used to immobilise the axial imidazole ligand **5**, which is a good substitute for the natural cysteine thiolate ligand. The distance between the porphyrin and the substrate or ligand can be tuned by using either molecule **3** or **4**. In Section 3.2.1 the synthetic approaches towards both compounds will be discussed.

3.1.3 Bipyridine linked CD-dimer as cofactor component in a Cytochrome P-450 mimic.

To achieve a well-controlled two-electron flow which minimises the non-productive generation of water we designed the CD-dimer **7**, shown below.



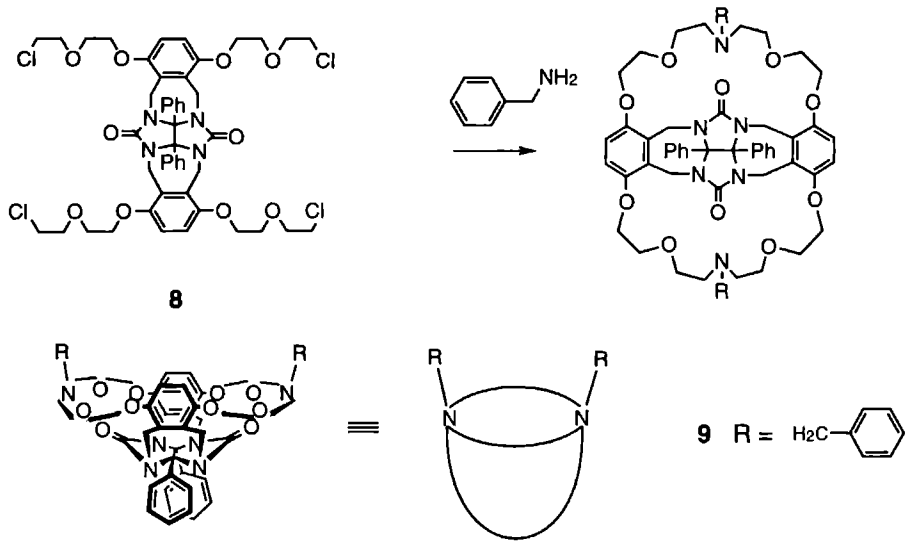
7

A manganese porphyrin can be encapsulated by **7** to form a 1:1 complex in a similar way as depicted in Figure 3.1. In such a complex an efficient two-electron transfer between the rhodium centre and the manganese porphyrin can be expected. To avoid the overflow of electrons in this system, the reactions after the reduction of the porphyrin (activation of molecular oxygen and the epoxidation of the substrate) have to take place much faster than the porphyrin reduction itself. It is not unlikely that this will occur since the decomposition of the Rh(III)-formate complex into Rh(III)-hydride and carbon dioxide is the rate limiting step in the catalytic cycle.[18] Also the degradation of the porphyrin as a result of μ-oxo dimer formation will be suppressed by using CD-dimer **7**.

In Section 3.2.2 the synthesis of dimer **7** will be described and in Section 3.3.2 the use of this compound as cofactor component in the epoxidation of nerol.

3.2 Synthesis of cyclodextrin dimers

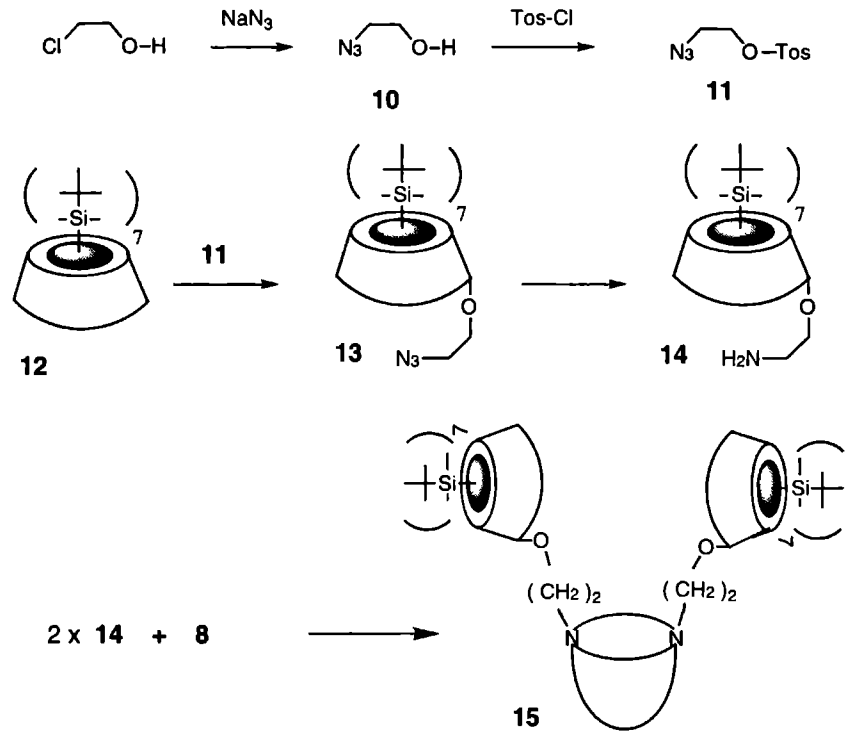
3.2.1 Bis-cyclodextrin modified basket molecule



Scheme 3.3

Previous work in our group has shown that basked-shaped molecules of type **9** can be synthesised as is depicted in Scheme 3.3. The building block **8** [19] is easily closed with amines, e.g. with benzylamine as in the case of compound **9**.[20] Our first synthetic strategy, therefore, involved the synthesis of an amine-functionalised CD derivative which can be reacted with compound **8**. This route will lead to molecule **15** as is shown in Scheme 3.4.

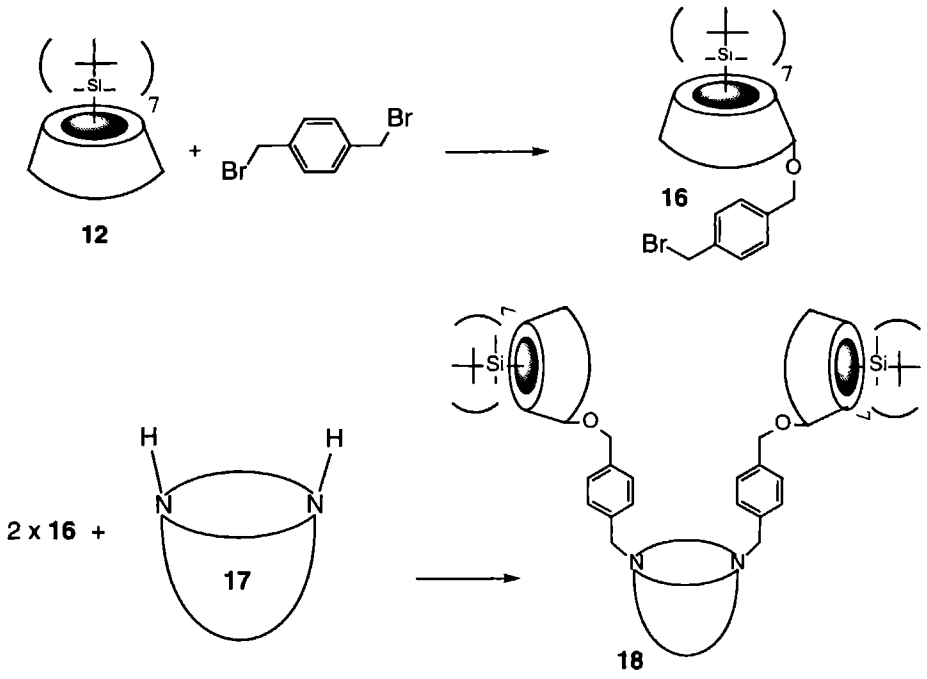
Our synthesis started with compound **10** which was obtained from 2-chloroethanol and sodium azide in 50% yield using a modified literature procedure.[21] The resulting alcohol was tosylated in pyridine to yield molecule **11** in 32% yield (not optimised). Molecule **11** was reacted with CD derivative **12**, which was first deprotonated at the hydroxyl group located at C-2 with sodium hydride, to give the azide-functionalised cyclodextrin **13** in 27 % yield after purification by repeated column chromatography. We found that the deprotonated cyclodextrin **12** did not react with alkyl halides. It is essential, therefore, to use the tosyl functionality as leaving group in the reaction step from **12** to **13**. The azide group of molecule **13** was reduced with tri-*n*-butyltin hydride in the presence of 2,2'-azobisisobutyronitrile (AIBN).[22] This method was much faster and provided higher yields than reduction with H_2 and palladium on carbon. The ring-closure reaction between amine **14** and the tetrachloro compound **8** under standard reaction conditions (acetonitrile, reflux, three equivalents of amine, NaI, several



Scheme 3.4

days)[19] did not result in the formation of new products, neither by applying prolonged reaction times nor by using increased amounts of **14**. The ring closure reaction therefore was tried at high pressure (12 kbar) in 1,2-dichloroethane and with ethyldiisopropyl amine as the base. In this case the formation of a mixture of approximately 5-10 % of new products was observed, which could not be purified. Also the exchange of the four chlorine atoms of **8** for iodine atoms before the reaction with **14**, did not lead to the formation of **15** under high pressure. These results are unexpected since several aminoalcohols react readily with compound **8** under standard reaction conditions.[27] The amine group did react, however, with the more reactive dansyl chlorides (Chapter 5). Apparently, it is not possible for the amine functionality of compound **14** to substitute the chlorine or iodine atoms of compound **8**. A possible explanation for this behaviour might be the steric hindrance caused by the large CD moiety. Since the method described above did not lead to the desired product **15**, a different synthetic strategy was followed to synthesise compound **18**.

This strategy involved the preparation of the basket-shaped compound **17**, which can be obtained by debenzylation of compound **9**. Compound **17** can react with benzylic bromides,[26] e.g. CD-derivative **16** to give the desired compound **18**.

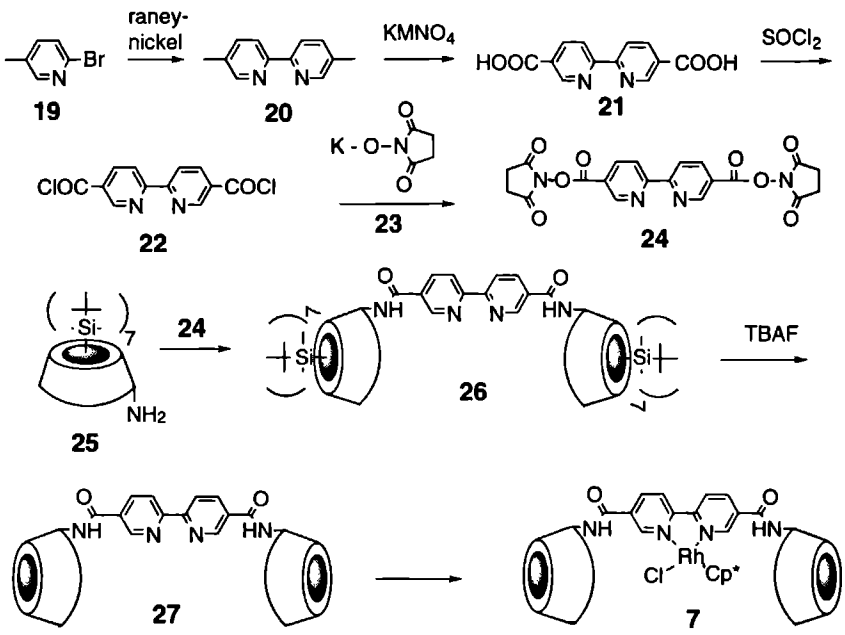


Scheme 3.5

Basket-shaped compound **17** was synthesised as described by Sijbesma.[19] To obtain the mono-functionalised CD **16**, the silylated CD **12** was deprotonated with NaH followed by reaction with an excess of α,α'-dibromo-*p*-xylene (Scheme 3.5). Compound **16** was isolated in 27% yield after repeated purification by column chromatography. This compound was reacted with the aza crown ether **17** to yield the CD dimer **18**. The purification of this dimer appeared to be very troublesome. Repeated column chromatography under conditions that are normally used to obtain pure diphenylglycoluril derivatives [27,15] yielded product **18** which was only 90% pure according to TLC. The use of reversed-phase chromatography did not improve the purity of this compound.

Since the target molecule could not be obtained in a pure form, we decided to focus on the synthesis of a bipyridine-linked CD-dimer, which will be presented in the next section.

3.2.2 Synthesis of bipyridine-linked CD-dimer



Scheme 3.6

The synthesis of dimer **7** is presented in Scheme 3.6. It started with the coupling of two molecules of 2-bromo-5-methylpyridine (**19**) using a stoichiometric amount of Raney-nickel. Subsequent reaction of the resulting nickel complex with ammonia yielded 5,5'-dimethylbipyridine **20** in 74% yield after purification. Oxidation of this compound with potassium permanganate yielded the corresponding diacid in 73% yield. Subsequent reaction with thionyl chloride yielded the diacid chloride **22** which was not purified. This compound was esterified using the potassium salt of *N*-hydroxysuccinimide (**23**) to give the active diester **24**. Cyclodextrin dimer **26** was obtained by reaction of this active ester with the monoamino-functionalised cyclodextrin **25** in refluxing THF. Purification by column chromatography yielded compound **26** in 50% yield. Desilylation was achieved by treatment with tetrabutylammonium fluoride in refluxing THF followed by repeated precipitation (yield 67%). In this way pure, water soluble, CD dimer **27** was obtained. This dimer was reacted with $[Cp^*RhCl(\mu\text{-}Cl)]_2$ in DMF to form compound **7**. The rhodium complex was precipitated from the reaction mixture by addition of diethyl ether (95% yield). The product was characterised by NMR, FAB-MS and elemental analysis.

3.3 Cytochrome P-450 mimic

3.3.1 Reduction of porphyrins

To investigate if the system described in Section 3.1.3 could be used for the epoxidation of olefins, we first studied the reduction of manganese porphyrins by the rhodium complex **7**. The reduced form of a porphyrin molecule is known to be oxidised very rapidly by molecular oxygen.[28] Therefore all measurements were performed under argon. Rhodium complex **7** was added to a solution of porphyrin **1b** in formate buffer at pH 7.3. In the UV/vis spectra a decrease of the absorption bands of the Mn(III) species took place while at the same time new bands of the Mn(II) species appeared. In Figure 3.2 the decrease of the absorption band of the Mn(III) porphyrin (466 nm) is plotted as a function of time for three different rhodium/ porphyrin ratios.

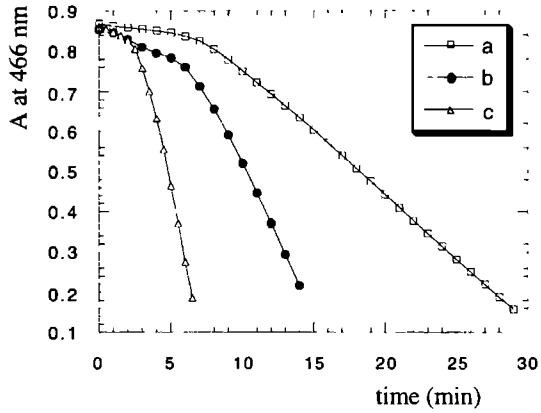


Figure 3.2 *Absorption changes at 466 nm (Mn (III)porphyrin **1b**) after the addition of cyclodextrin dimer 7 as a function of time at different rhodium/porphyrin molar ratios: (a) 1/16; (b) 1/8; (c) 1/2.7. [**1b**]=9.9 x 10^{-6} M, [NaHCOO]=0.5 M, [KH_2PO_4] = 0.1 M, buffered at pH 7.3, T=25 °C.*

From this plot it can be seen that the reduction of the porphyrin is accompanied by an induction period of several minutes which is probably caused by a small amount of oxygen that could not be removed by purging the system with argon (at the concentrations used, 0.4 µl oxygen is as much as one equivalent). After this induction period the decrease of the concentration of the Mn(III) species is linear with time. The reduction rate appeared to be independent of the concentration of the porphyrin (results not shown), indicating a zero-order reaction in porphyrin concentration. After the reduction was complete, contact with air resulted in a rapid

reoxidation of the Mn(II) species to the Mn(III) species showing that the reduction reaction is reversible. Figure 3.3 shows the results of experiments carried out at different concentrations of dimer **7**. This figure reveals that a clear relationship exists between the reduction rate of porphyrin **1b** and the concentration of dimer **7**, indicating a first order reaction in rhodium. The slope of this curve gives the turnover frequency (mol porphyrin per mol rhodium per second) which amounts to $(9.6 \pm 0.3) \times 10^{-3}$ s^{-1}. This value is slightly lower than the values reported by van Esch et al. [29] for the same porphyrin which was reduced by a rhodium complex incorporated in polymerised vesicle bilayers (approx. 16×10^{-3} s^{-1}). A plausible explanation for this difference is the high local concentration of formate anions at the surface of the positively charged vesicles in the system used by van Esch.[7]

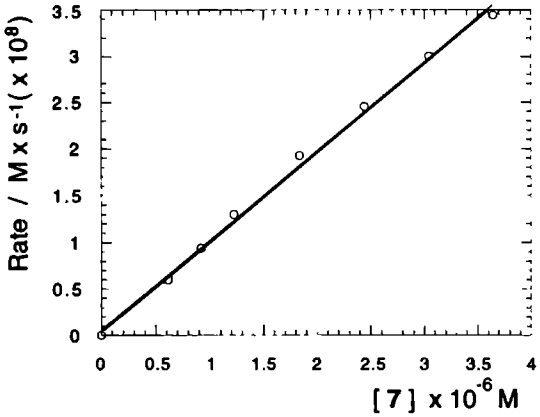


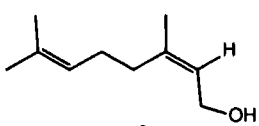
Figure 3.3. *Rate of reduction of compound 1b as a function of the concentration of 7. For reaction conditions see Figure 3.2.*

In the epoxidation reactions we used porphyrin **1a** instead of **1b** since we expected that the former, less polar porphyrin would bind much stronger into the CD dimer **7.** We investigated, therefore, if this porphyrin could also be reduced by the rhodium complex **7**. Since porphyrin **1a** does not dissolve in an aqueous buffer, we used an aqueous ethanol solution (1/1, v/v) for the determination of the reduction rate. Under these conditions we found turnover frequencies of $(2.3 \pm 0.3) \times 10^{-3}$ and $(1.9 \pm 0.2) \times 10^{-3}$ s^{-1} for porphyrins **1b** and **1a**, respectively. Apparently, both porphyrins are reduced at the same rate. This is consistent with the fact that the rate limiting step in the reduction process is the conversion of the Rh(III)-formate complex into a Rh(III)-hydride species and CO_2.[18] Since this process is the same for both porphyrins no significant difference in turnover frequencies is expected. The water soluble porphyrin **1b** is reduced four times slower in the aqueous ethanol solution than in buffered water. This is

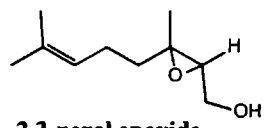
probably caused by changes in the stability of the Rh(III)-formate complex. Also the reduction potentials may be different in these two media.

3.3.2 Epoxidation experiments

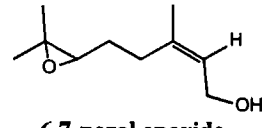
In a subsequent series of experiments it was tested whether a catalytic system containing porphyrin **1a** and rhodium complex **7** was capable of catalysing the oxidation of nerol by molecular oxygen. We used the same solvent and buffer as described by Tabushi et al.,[30,10] since they showed that nerol epoxides are stable for at least 48 h at room temperature under these conditions. Also, the reaction of the Mn(V)-oxo species with ethanol, which may give $Mn(IV)(OEt)_2$-species,[31] does not occur or does not influence the catalytic activity of the porphyrin in this solvent mixture.



nerol



2,3-nerol epoxide



6,7-nerol epoxide

In a typical reaction, 2.0 ml of an aqueous ethanol (1/1, v/v) solution containing 0.25 M NaHCOO, 0.05 M phosphate buffer, (pH 7.3), 0.5 mM porphyrin **1a**, 0.5 mM rhodium complex (either **7** or model compound **2**) and 50 mM nerol was stirred at 50 °C under an oxygen atmosphere ($p(O_2)$ = 760 mm Hg) with or without N-methylimidazole (0.05 mM) present. Analysis of the reaction mixture by GLC revealed that oxidation of nerol occurred. The oxidation products were identified by comparing their retention times with those of reference compounds, which were synthesised separately (see Experimental section). After the alkene conversion had stopped, the turnover numbers (mol products per mol catalyst) were determined. In the absence of porphyrin, rhodium complex, sodium formate or dioxygen no significant formation of oxidation products was detected after four days while the alkene was still present. In Table 3.1 the results of the catalytic experiments are summarised. They show that the catalyst system based on CD-dimer **7** is able to catalyse the epoxidation of nerol. This substrate is converted into 2,3-nerol epoxide and 6,7-nerol epoxide in a constant ratio of approximately 1:9 indicating that the epoxidation is regioselective. This is consistent with observations made by Tabushi et al. who found the same ratio in a similar system using porphyrin **1b** and N-methyldihydronicotinamide as the reducing agent in an aqueous ethanol solution.[10] Tabushi et al. used benzoic anhydride to facilitate the formation of the Mn(V)-oxo complex.[9] The presence of this anhydride in our system (5×10^{-2} M) did not influence the turnover numbers nor did it affect the reaction time. Formation of the diepoxide was negligible.

Table 3.1 *Catalytic activity of Cytochrome P-450 model systems in the epoxidation of nerol* [a]

Entry no	Rh-complex	[*N*-MeIm] x 10^3 M	TO[b] (± 1)	Reaction time (h)[c]
1	**2**	-	3	24
2	**2**	50	3	96
3	**7**	-	9	24
4	**7**	50	13	96

[a] *Reaction conditions 2 ml of an aqueous ethanol solution (1/1, v/v) containing 0 25 M NaHCOO and 0 05 M* KH_2PO_4, *buffered at pH 7 3, [7] or [2] = 0 5 mM , [1a] = 0 5 mM, [nerol] = 50 mM, oxygen atmosphere (*$p(O_2)$ *= 760 mm Hg), T = 50 °C N-MeIm = N-methylimidazole* [b] *TO turnover number = mol of products formed per mol of catalyst determined after alkene conversion had stopped* [c] *After this period no further alkene conversion was observed, the porphyrin had been decomposed*

In a control experiment we tested the stability of the nerol epoxides under the same experimental conditions as used in the catalytic experiments, but in the absence of rhodium complex This experiment showed that decomposition of the epoxides and alkene was less than 15% in four days For comparison we also determined the turnover numbers (Table 3 1) of the reference compound **2** (Scheme 3 1) It can be seen in Table 3 1 that the cyclodextrin-modified rhodium complex **7** is approximately three to four times more effective in the epoxidation reaction than the unmodified complex **2** Steckhan et al showed that the efficiency of 2,2'-bipyridine rhodium complexes in reduction reactions is decreased by electron withdrawing substituents on the bipyridine ligand [32] Since the bipyridine unit in molecule **7** is functionalised with two electron withdrawing amide groups at the 5,5'-positions it can be expected that the reduction of porphyrins, and therefore the subsequent epoxidation of alkenes with this complex is *less* effective than with a system that uses compound **2** As can be seen in Table 3 1, however, the epoxidation reaction is *more* effective when compound **7** is applied as the catalyst A possible explanation for this difference in turnover numbers may be that the reduced manganese(II) porphyrin has the possibility to dimerise or to aggregate in the absence of the CD units Such dimers or aggregates are catalytically less active [33] This aggregation is suppressed by the encapsulation of the porphyrin into the CD cavities [34,35] The formation of an inclusion complex may also lead to an easier electron transfer reaction between the rhodium and the porphyrin All these processes will result in higher turnover numbers when compound **7** is used as the cofactor reductant

When an O-O bond has to be broken in the catalytic cycle the presence of *N*-methylimidazole (*N*-MeIm) as a cocatalyst in the system may be favourable [36] To investigate the influence of

N-MeIm in our system, the reaction was carried out with and without *N*-MeIm present. The porphyrin employed in the reaction appeared to be totally degraded after 24 h if no *N*-MeIm was present (Table 3.1, entries 1 and 3). With *N*-MeIm the lifetime of the porphyrin increased, although after 96 h of reaction no porphyrin could be detected anymore (entries 2 and 4). The destruction of porphyrins is believed to be initiated by the formation of a porphyrin μ-oxo dimer.[11] This dimer formation is suppressed in the presence of *N*-MeIm, which shields at least one side of the porphyrin ring by coordinating to the manganese centre. As a result, the life time of the porphyrin will be longer. This may explain the slightly higher turnover number found for compound **7** in the presence of *N*-MeIm. When **2** was used as the reductant component, the expected increase in turnover number using *N*-MeIm was not observed, probably due to the relatively large experimental error.

From the results presented above, we may conclude that the use of CD dimer **7** leads to a more efficient catalytic system when compared to the free rhodium complex **2**. The rate of epoxide formation (13 turnovers in 4 days) is still very small compared to similar systems described by Tabushi et al. (85 turnovers in 2 days [30] and 45 turnovers in 5 min.[10]) and by Schenning in our group (360 turnovers 1 h [7]). This might be caused by shielding of the active Mn(V)-oxo species by the cyclodextrin moiety, which prevents the alkene to come in close proximity of the oxidising species. Protons and electrons can approach the porphyrin more easily, favouring the non-productive reaction.

3.4 Concluding remarks

The synthesis of CD dimers that are connected by a basket-shaped molecule based on diphenylglycoluril is possible via the aza crownether basket molecule **17**. The purification of the resulting compound **18** was difficult and a product with only 90 % purity could be achieved. Recent studies in our group have shown that basket-shaped diphenylglycoluril molecules are able to bind substrates in aqueous media.[37] This feature opens the way for future applications of bis-CD modified basket molecules in aqueous media. Further attempts to purify compound **18**, therefore, should be undertaken.

The results obtained with the Cytochrome P-450 mimic in which the cyclodextrin modified rhodium complex **7** is used as a cofactor reductant are very preliminary. Many parameters important for describing the catalytic cycle were not investigated because the overall epoxide formation was too slow. This fact and the probable degradation of the porphyrin make that the catalytic system is not of interest for future application in industrial processes.

Although the reaction conditions were not optimised, the result that the present catalyst system is able to catalyse the epoxidation of nerol is interesting from a fundamental point of view The catalytic system containing the CD dimer displays higher turnover numbers than a system in which the CD moieties are absent This is probably due to the encapsulation of the porphyrin by one or two of the CD moieties and to a more efficient electron transport between the rhodium and the manganese centres Knowledge of the geometry of the complex between the cofactor reductant and the porphyrin is important for further improvement of the Cytochrome P-450 mimic Studies to determine this geometry are described in the next chapter

3.5 Experimental

General: See Section 2 6 for experimental details on syntheses
Eluents used in chromatography were mixtures (v/v) of ethyl acetate, ethanol and water (A (100 4 2), B (100 8 4), C (100 14 8), D (100 30 16), F (100 2 1)) 2-Bromo-5-methylpyridine (**19**) was prepared from commercially available 2-amino-5-methylpyridine in 92% yield using a procedure described by Adams et al for 2-bromo-3-methylpyridin [38] The Raney-nickel was dried before use as follows water was removed and the solid was repeatedly washed with ethanol under argon twice with ethanol, technical grade and twice with ethanol, analytical grade The last traces of ethanol were removed *in vacuo* Dichloropentamethylcyclopentadienylrhodium dimer, $[Cp^*RhCl(\mu\text{-}Cl)]_2$, was synthesised as described by Maitlis et al from hexamethylbicyclo[2 2 0]hexadiene and $RhCl_3\ 3H_2O$ [39] The potassium salt of *N*-hydroxysuccinimide was prepared as described before [40] Compounds **8**, **9** and **17** were prepared as described by Sijbesma [19, 20] Cyclodextrin derivatives **12** and **25** were prepared as described in Chapter 2 The ^{1}H-NMR spectra of CD containing compounds were usually obtained after H-D exchange using either CD_3OD or D_2O

Catalysis:
Porphyrin reduction experiments were carried out in an aqueous phosphate buffer (0 1 M, buffered at pH 7 3) In a typical experiment 2 ml of the formate buffer containing 0 5 M of sodium formate and 1 x 10^{-5} M of porphyrin was placed in a quartz cuvette and degassed by bubbling argon through the solution for 15 min The cuvette was closed with a rubber septum and placed in the thermostatted cuvette-holder of the spectrophotometer After addition of a stock-solution of the rhodium complex with a syringe (5-25 µl), the course of the reaction was followed by measuring the change in absorbance of the porphyrin at 466 nm (**1b**) or at 464 nm (**1a**)
Catalytic experiments were performed as follows an aqueous buffer containing 0 5 M sodium formate/ 0 1 M phosphate, pH 7 3 was mixed with an equal volume of ethanol (analytical grade) To this solution were added stock solutions of the rhodium-complex, porphyrin, substrate and ligand The reaction was monitored on a Varian 3700 gas chromatograph with flame ionisation detector The substrates and reaction products were separated on a CP-SIL 5CB capillary column (25m x 0 25 mm ID, df = 0 25 µm, using a temperature program) The detector signal was integrated using a HP 3390A integrator For identification of the products, the epoxides of nerol were synthesised as described below

2,3-Nerol epoxide and **6,7-nerol epoxide**

These reference compounds were obtained by the oxidation of nerol: 0.9 g of nerol and 1.5 g (1.5 equiv.) of *m*-chloroperoxybenzoic acid were dissolved in 40 ml of dichloromethane and stirred for 3 h at room temperature. After washing the reaction mixture with aqueous 1M NaOH and with brine, the solvent was removed *in vacuo* and the crude oil was purified by column chromatography (silica 60, 100 g, eluent: ethyl acetate/hexane, 1/2, v/v). In this way the two regio isomers were separated and obtained as colourless oils which were pure according to GLC. Assignment of the chemical structure was made using reported ^{1}H-NMR data of 2,3-nerol epoxide.[30]

2,3-Nerol epoxide: Yield: 111 mg (14%). R_f(ethyl acetate/hexane, 1/1, v/v) = 0.51. ^{1}H-NMR ($CDCl_3$, 90 MHz) : 5.05 (m,1H,C=C*H*), 3.8-3.5 (m, 2H, CH_2-OH), 2.90 (m, 1H, C*H*), 2.1 (m, 2H, CH_2-C=C), 1.7 and 1.6 (2xs, 6H, 2x(C=C)-CH_3), 1.7-1.3 (m+s, 5H, CH_2.CH_2 and COC-CH_3).

6,7-Nerol epoxide: Yield: 260 mg (20%). R_f(ethyl acetate/ hexane, 1/1, v/v) =0.34. ^{1}H-NMR ($CDCl_3$, 90 MHz) : 5.35 (m,1H,C=C*H*), 3.95 (d, 2H, CH_2-OH), 2.60 (t, 1H, COC-*H*), 2.1 (m, 2H, CH_2-C=C), 1.7-1.4 (m+s, 5H, CH_2.CH_2 and C=C-CH_3), 1.2 (2xs, 6H, 2x(COC)-CH_3).

2-Azido-ethanol (10)

This compound was synthesised using a procedure described by Boyer et al.[21] which we modified as follows. 2-Chloroethanol (23.5 g) was added to 25.2 g (1.33 equiv.) of sodium azide. This suspension was heated at 90 °C for 120 h. The reaction mixture was poured into dichloromethane (100 ml) and the sodium salts were removed by filtration. After evaporation of the dichloromethane *in vacuo* the product was purified by distillation yielding a colourless oil. Yield: 12.46 g (50%). Bp 69 °C (20 mm Hg). ^{1}H-NMR ($CDCl_3$, 90 MHz) : 3.75 (t,2H,O-CH_2), 3.33 (t,2H,N_3-CH_2), 2.95 (s,1H,O-*H*). IR: 2110 (N_3). CI-MS (m/e): 88 (M+1), 175 (2M+1).

1-Tosyloxy-2-azido-ethane (11)

To a solution of 7.35 g of 2-azidoethanol (**10**) in 50 ml of pyridine was added at 0 °C 17.96 g (1.08 equiv.) of tosyl chloride. After stirring for 24 h at room temperature the reaction mixture was concentrated *in vacuo*, the product dissolved in 75 ml of dichloromethane, and the solution washed with 1M aqueous HCl (twice), with a saturated solution of $NaHCO_3$, and with brine. After drying the organic layer over $MgSO_4$ and evaporation of the solvent, 9.5 g of crude product was obtained, which was purified by column chromatography (silica 60, 90 g, eluent: petroleum ether 60-80/ethyl acetate, 4/1, v/v) yielding **11** as a colourless oil. Yield: 6.68 g (32%). ^{1}H-NMR ($CDCl_3$, 90 MHz) : 7.7 and 7.3 (2xd, 2x2H, Ar-*H*), 4.1 (t,2H,O-CH_2), 3.4 (t,2H,N_3-CH_2), 2.4 (s,3H,CH_3). IR: 2105 (N_3). CI-MS (m/e): 242 (M+1), 483 (2M+1). Anal. Calcd for $C_9H_{11}N_3O_3S$: C, 44.80; H, 4.60; N, 17.42; S, 13.29. Found : C, 44.86; H, 4.64; N, 17.08; S, 13.53.

Mono-2-O-(2-azido-ethyl)-heptakis-6-O-(*tert*-butyldimethylsilyl)-β-CD (13)

To a solution of 14.02 g of dried (95 °C, 0.1 mm Hg, 5 h) cyclodextrin derivative **12** in 180 ml of dry THF was added 525 mg (3.0 equiv.) of cleaned NaH. The suspension was stirred for

at least 17 h at room temperature and 1 h at reflux temperature Subsequently, 1 67 g (0 96 equiv) of compound **11** was added After 5 h of reaction, TLC (eluent C) showed the formation of two new products The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in ethyl acetate The solution was washed with water/brine (1/1, v/v) and dried over $MgSO_4$ After removal of the solvent *in vacuo*, 16 g of crude product was obtained which was subjected twice to column chromatography (1 4 kg silica, a gradient was used going from eluent F to eluent D) to give compound **13** as a white solid In this way also 6 2 g of pure starting material **12** was recovered Yield of **13** 3 97 g (27%, or 47% based on the consumed amount of **12**) Mp 253-258 °C R_f(C)= 0 47 ^{1}H-NMR (C_6D_6) 5 32-4 93 (m, 7H, *H*-1), 4 45-3 45 (42H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6), 3 05 (br m, 2H, C*H*$_2$-N_3), 1 35-0 80 (m, 63H, C*H*$_3$-*C*), 0 3-0 17 (m, 42H, C*H*$_3$-Si) ^{13}C-NMR (C_6D_6) 104 1-102 0 (*C*-1), 83 9-81 2, 75 1-73 2 (*C*-2, *C*-3, *C*-4 and *C*-5), 73 0 (*C*H$_2$-CH$_2$-N_3), 63 3-62 8 (*C*-6), 51 6 (CH$_2$-*C*H$_2$-N_3), 26 9 (CH$_3$-*C*), 19 3 (*C*-(CH$_3$)$_3$), -4 2 (*C*H$_3$-Si) FAB-MS (m/e) 2025 (M+Na) and 2135 (M+Cs) Anal Calcd for $C_{86}H_{171}O_{35}N_3Si_7$ C, 51 55, H, 8 60, N, 2 10 Found C, 51 63, H, 8 51, N, 2 05

Mono-2-O-(2-amino-ethyl)-heptakis-6-O-(*tert*-butyldimethylsilyl)-β-CD (14)

CD derivative **13** (300 mg) and 12 5 mg of 2,2'-azobisisobutyronitrile (AIBN) (0 62 equiv) were dissolved in 6 ml of THF After the addition of 76 mg of tri-*n*-butyltin hydride (2 1 equiv) the reaction mixture was refluxed for 2 h After removal of the solvent *in vacuo* the crude product was dissolved in ethyl acetate and the solution washed with water/brine (1/1, v/v) and brine The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* The product was obtained as a white solid after column chromatography (40 g silica, eluent D) Yield 220 mg (74%) Mp 231 °C (dec) R_f(D)= 0 30 ^{1}H-NMR ($CDCl_3$/CD_3OD, 1/1, v/v) 4 75 and 4 73-4 50 (d and m, 1H+6H, *H*-1), 3 75-2 75 (m, 46H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6 and 2x*CH*$_2$-spacer), 0 58 (s, 63H, C*H*$_3$-*C*), -0 25 (s, 42H, C*H*$_3$-Si) ^{13}C-NMR ($CDCl_3$/CD_3OD, 1/1, v/v) 101 8-101 5 and 99 3 (*C*-1), 80 9-80 2, 72 9-71 4 (*C*-2, *C*-3 *C*-4 and *C*-5), 66 6 (*C*H$_2$-O), 61 4-61 2 (*C*-6), 38 8 (*C*H$_2$-NH$_2$), 25 1 (CH$_3$-*C*), 17 6 (*C*-(CH$_3$)$_3$), -5 9 (*C*H$_3$-Si) FAB-MS (m/e) 1978 (M+1) and 2000 (M+Na) Anal Calcd for $C_{86}H_{173}O_{35}NSi_7$ 5H_2O C, 49 95, H, 8 92, N, 0 68 Found C, 49 77, H, 8 54, N, 0 71

5,5'-Dimethyl-2,2'-bipyridine (20)

The synthesis of compound **20** was performed using a procedure of Breitmaier et al ,[41] which was modified as follows Under argon, 40 95 g of 2-bromo-5-methylpyridine (**19**) was dissolved in 150 ml of toluene This solution was added to a suspension of dried Raney-nickel (8 0 g) in 50 ml of toluene After refluxing for 3 days, the solution was cooled to room temperature and the precipitate was collected by filtration The filtrate was rinsed with toluene to remove unreacted 2-bromo-5-methylpyridine The green residue was dried *in vacuo* yielding 45 5 g (96%) of **20**.Ni(Br)$_2$ An amount of 25 4 g of this product was dissolved in 300 ml of aqueous HCl (6 M) and an aqueous 25% NH_3-solution was added until the former solution was basic Many (approx 50 !) extraction's with $CHCl_3$ were necessary to obtain 10 g of crude product Compound **20** was obtained as white needles after purification by column chromatography (Silica 60, $CHCl_3$) followed by recrystallisation from ethanol Yield 8 7 g (74%) Mp 114-5 °C (lit[25] 114 5-115 °C) ^{1}H-NMR ($CDCl_3$, 90 MHz) 8 40 (s,2H,Ar-*H*-6), 8 25 (d,2H,Ar-*H*-3), 7 5 (d,2H,Ar-*H*-4), 2 30 (s,6H,C*H*$_3$) Anal Calcd for $C_{12}H_{12}N_2$ C, 78 23, H, 6 56, N, 15 20 Found C, 78 14, H, 6 82, N, 14 98

[2,2'-Bipyridine]-5,5'-dicarboxylic acid (21)

Compound **20** was oxidised with $KMnO_4$ following a procedure described by Case,[23] which was modified as follows A mixture of 30 8 g of $KMnO_4$ and 5 6 g of compound **20** in 500 ml of water was heated (70 °C) for 24 h After filtration of the reaction mixture and washing the brown filtrate with 50 ml of aqueous 1M NaOH, the combined water fractions were collected and washed three times with $CHCl_3$ to remove unreacted **20** The aqueous solution was neutralised with HCl (2M) and concentrated to 300 ml After acidifying to pH 6 the light blue precipitate was collected by centrifugation, washed with ethanol (three times) and dried *in vacuo* Yield 5 25 g (71%) Mp > 350 °C IR 1680 (CO), 1590 (Ar) ^{1}H-NMR (D_2O,NaOD) 8 9 (s,2H,Ar-*H*-6), 8 2 (d,2H,Ar-*H*), 7 7 (d,2H,Ar-*H*)

[2,2'-Bipyridine]-5,5'-dicarboxylic acid bis(*N*-hydroxysuccinimide) ester (24)

Compound **21** (2 17 g) was dissolved in 40 ml of $SOCl_2$ and refluxed for 24 h After removal of the excess of $SOCl_2$ *in vacuo*, the resulting diacid chloride **22** was directly dissolved in CH_2Cl_2 and 5 0 g (3 7 equiv) of the potassium salt of *N*-hydroxysuccinimide was added After 6 h stirring at room temperature, the reaction was quenched by addition of 200 ml of an aqueous saturated solution of $NaHCO_3$ The aqueous layer was extracted 15 times with CH_2Cl_2 (150 ml) The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* yielding 2 4 g of crude product Removal of unreacted compound **21** was achieved by dissolving the crude product in 1 l of CH_2Cl_2 followed by washings with aqueous NaOH (0 5 M), a saturated solution of $NaHCO_3$ and with brine The resulting compound was recrystallised from acetonitrile to give light yellow crystals Yield 1 7 g (43%) Mp 300 °C (dec) ^{1}H-NMR (DMSO-d6) 9 40 (s,2H,Ar-*H*), 8 73 (s,4H,Ar-*H*), 2 94 (s,8H,CH_2) Anal Calcd for $C_{20}H_{14}N_4O_8$ C, 54 80, H, 3 22, N, 12 78 Found C, 55 18, H, 3 27, N, 12 58

***N,N'*-Bis[mono(3-deoxy)heptakis(6-O-*tert*-butyldimethylsilyl)-β-CD]-5,5'-dicarboxamide-2,2'-bipyridine (26)**

To a solution of 2 45 g of dried (1h, 40 °C) monoamino-functionalised cyclodextrin **25** in 30 ml of THF was added 277 mg (1 equiv) of compound **24** After addition of two drops of triethylamine the reaction mixture was refluxed for 40 h After removal of the organic solvents *in vacuo*, the resulting solid was dissolved in ethyl acetate The solution was washed with NaOH (twice, 1M) and brine (twice), dried over $MgSO_4$, and concentrated *in vacuo* to yield 2 5 g of crude product Compound **26** was purified by column chromatography (300 g silica, eluent A) yielding a white solid Yield 1 15 g (45%) Mp > 350 °C ^{1}H-NMR ($CDCl_3$) 9 5-7 8 (5 x br s, approx 6H,Ar-*H*), 5 1-4 6 (br m, 14H,*H*-1), 4 4-3 2 (br m, H-CD-unit), 0 9-0 6 (br s,CH_3-C), 0 2-(-0 2) (br s,CH_3-Si) The peaks in the NMR-spectra of this compound were broad which may be due to the presence of more than one conformer in solution ^{13}C-NMR ($CDCl_3$) 168, 156, 149, 147 5, 138, 137 5, 129 5, 124 (Ar-*C* and *C*O, more signals than expected probably due to the presence of more conformers), 104 3-100 5 (*C*-1), 83 1-71 7 (*C*-2, *C*-3, *C*-4 and *C*-5), 62 7-61 4 (*C*-6), 26 7-25 8 (*C*H_3-*C*), 18 3 (*C*-$(CH_3)_3$), -4 8-(-5 5) (*C*H_3-Si) Anal Calcd for $C_{180}H_{342}N_4O_{70}Si_{14}$ $4H_2O$ C, 52 12, H, 8 50, N, 1 35 Found C, 52 12, H, 8 55, N, 1 29

N,N'-Bis[mono(3-deoxy)-β-CD]-5,5'-dicarboxamide-2,2'-bipyridine (27)

Compound **26** (640 mg) was dried (1 h, 0 05 mm Hg, 40 °C) and dissolved in 15 ml of THF After addition of 2 4 ml of a 1 0 M stock solution of TBAF in THF (15 5 equiv), the reaction mixture was refluxed for 24 h After concentration *in vacuo*, the crude product was dissolved in a minimum amount of water This solution was poured into ethanol (analytical grade) and the product was collected by centrifugation Repeating this procedure twice afforded pure compound **27** as a slightly purple precipitate Yield 260 mg (67%) Mp 325-327 °C (dec) IR 1625, 1510 (amide I and II, and bipyridine C=C) FAB-MS (m/e) 2477 (M+1) ^{1}H-NMR (D_2O) 8 90 (s,2H,Ar-*H*), 8 24 (br s,2H,Ar-*H*), 8 13 (br s,2H,Ar-*H*), 5 08-4 98 (m, 14H,*H*-1), 4 45, 4 21 (2xbr s,2x2H) and 4 07-3 49 (2 x m, approx 80H, *H*-CD-unit) ^{13}C-NMR (D_2O) 168 9 (*C*=O), 157 7 (bipy-*C*-2), 149 5 (bipy-*C*-6), 138 5 (bipy-*C*-4), 131 4 (bipy-*C*-5), 123 3 (bipy-*C*-3), 105 0 and 103 2-102 5 (*C*-1), 82 4, 82 1, 81 9, 81 4, 74 4-72 6 and 71 07 (*C*-2, *C*-3, *C*-4 and *C*-5), 61 5-61 1 (*C*-6), 53 0 (*C*-3^a) Anal Calcd for $C_{96}H_{146}N_4O_{70}$ $10H_2O$ C, 43 41, H, 6 30, N, 2 11 Found C, 43 49, H, 6 31, N, 2 09

[(η^5-Pentamethylcyclopentadienyl)(*N,N'*-bis[mono(3-deoxy)-β-CD]-5,5'-dicarboxamide-2,2'-bipyridine)chlororhodium] chloride (7)

Introduction of the metal centre in compound **27** was performed using a modified procedure of Ziessel et al [24] The reactions were carried out under an argon atmosphere A solution of **27** (100 mg) in 1 ml of DMF was added to a solution of 12 5 mg of $[Cp^*RhCl(\mu\text{-}Cl)]_2$ in 2 ml of DMF After stirring for 12 h at 40 °C the reaction mixture was poured into 50 ml of diethyl ether The precipitate was collected by centrifugation and washed twice with 25 ml of diethyl ether To remove the last traces of DMF the precipitate was dissolved in 1 ml of water and precipitated by addition of 50 ml of acetone The product was collected by centrifugation and dried *in vacuo* to give a yellow solid Yield 110 mg (95%) Mp 270 °C (dec) ^{1}H-NMR (D_2O) : 9 31, 9 26 and 8 64-8 56 (6H, Ar-*H*), 5 12-4 97 (m, 14H, *H*-1), 4 27-3 35 (4 x m, approx 84H), 1 74 (s, 15H, CH_3-Cp*) FAB-MS (-m/e) 2711 (M-2Cl-1), 2645 (M-RhCl-1), 2473 (M-$RhCl_2Cp^*$-1) Anal Calcd for $C_{106}H_{161}N_4O_{70}Cl_2Rh$ 14 H_2O C, 41 92, H, 6 27, N, 1 84 Found C, 41 99, H, 6 49, N, 1 89

Mono-2-O-(4-bromomethyl)benzyl-heptakis(6-O-*tert*-butyldimethylsilyl)-β-CD (16)

To a solution of 5 g of dried (2 h, 80 °C, 0 05 mm Hg) CD derivative **12** in 60 ml of dry THF was added 70 mg (0 9 equiv) of NaH (80% dispersion in oil) After stirring the reaction mixture for 20 h at room temperature, 3 75 g (5 5 equiv) of α,α'-dibromo-*p*-xylene (recrystallised from ethyl acetate) was added After 6 h stirring, the reaction mixture was concentrated *in vacuo*, the residue dissolved in ethyl acetate, the resulting solution washed with water/brine Further purification was achieved by repeated column chromatography (450 g silica, eluent A) A total amount of 2 1 g of starting material **12** was recovered and nearly pure (>95% according to TLC) product was obtained as a white solid Yield 1 47 g (27%, or 46% according to the consumed amount of **12**) Mp 270-271 °C (dec) ^{1}H-NMR ($CDCl_3$) 7 35 (m, 4H, Ar-*H*), 4 9-3 3 (approx 49H, *H*-1, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6 and CH_2-Ar), 0 9-0 75 (m, 63H, CH_3-*C*), 0 1-0 0 (m, 42H, CH_3-Si) ^{13}C-NMR ($CDCl_3$) 137 7, 137 5, 129 4 and 129 1 (aromatic *C*-), 103 0, 102 3-101 9, 101 0 (*C*-1), 82 0-81 7, 80 3, 79 5, 73 9-72 4 (*C*-

2, *C*-3 *C*-4 and *C*-5), 62.2-61.4 (*C*-6 and O-CH_2-Ar), 32.9 (CH_2-Br), 26.0-25.8 (CH_3-*C*), 18.3 (*C*-$(CH_3)_3$), -5.1 (CH_3-Si). FAB-MS (m/e): 2139.5 (M+Na), 2250.3 (M+Cs). Anal. Calcd for $C_{92}H_{175}O_{35}Si_7Br$: C, 52.18; H, 8.33. Found : C, 52.18; H, 8.17.

Bis-cyclodextrin modified basket molecule (18)

Cyclodextrin derivative **16** (212 mg), 43.8 mg of azacrown basket molecule **17** and 0.1 ml of triethylamine were dissolved in 3 ml of THF. After two days stirring at room temperature, the reaction mixture was concentrated *in vacuo* and the residue dissolved in chloroform. The resulting solution was washed with water and brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness to yield 180 mg of crude product. An attempt to purify this product by silica gel column chromatography (silica 60H, eluent: 10% (v/v) MeOH, 1% (v/v) triethylamine in $CHCl_3$) failed. A better purification was achieved using a silica column that was saturated with KBr (eluent 30% (v/v) ethanol in $CHCl_3$).[27] Compound **18** was obtained in 90% purity as a white solid. Yield: 70 mg (28%). Mp: 219-222 °C. ^{1}H-NMR ($CDCl_3$ after H-D exchange using D_2O) : 7.35 (br.s, 8H, Ar-*H*), 7.15 (br.s, 10H, Ar-*H*), 6.60 (br.s, 4H, Ar-*H*), 5.65 (br.d, 4H, NC*H*HAr), 5.0-4.8 (br.s, 14H, *H*-1), 4.25-3.25 (4 x m, approx. 120H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6, CH_2-xylene, CH_2-O, CH_2-N and NCH*H*Ar), 0.9-0.75 (m, approx. 126H, CH_3-*C*), 0.1-0.05 (m, approx. 84H, CH_3-Si) and several small signals resulting from impurities. The peaks in the NMR spectra ($CDCl_3$) were broad, probably due to the presence of more than one conformation in solution. FAB-MS (m/e): 4950 (M+1), 2914 (M - cyclodextrin-benzyl fragment). Anal. Calcd for $C_{232}H_{406}O_{80}N_6Si_{14}$: C, 56.26; H, 8.26; N, 1.70. Found : C, 55.96; H, 8.19; N, 2.14.

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CHAPTER 4

Binding Studies on Cyclodextrin Dimers

4.1 Introduction

Probably the most important property of cyclodextrins is their ability to form complexes with a variety of organic compounds. This property was used by Schardinger in 1911 when he isolated the cyclodextrins by forming their precipitates with alcohol, chloroform and ether.[1] It was only thirty years later that Freudenberg realised that these precipitates resulted from the inclusion of molecules *in* the CD-cavities.[2] This hypothesis was generally accepted, but could not be proven at that time. The direct proof of the existence of an inclusion complex was obtained by Hybl et al. in 1965 from X-ray diffraction data on the complex between α-cyclodextrin and potassium acetate.[3] From these data it was concluded that the acetate anions were located inside the cavity. The first direct evidence of an inclusion complex *in solution* was published in 1970. Demarco et al. [4] added aromatic guest molecules to aqueous solutions of cyclodextrins and observed that in the ^{1}H-NMR spectra of the latter compounds the resonances of the hydrogen atoms located inside the cavity were significantly shifted upfield due to shielding effects of the guest molecules, whereas the resonances of the hydrogen atoms at the outside of the CD molecule showed little effect. Many other techniques have been used since then to obtain direct or indirect proof of the inclusion of guest molecules. Among these are solubility studies, UV-vis, EPR-, circular dichroism and fluorescence spectroscopy, conductometry, potentiometry, microcalorimetry and chromatography.[5,6,7] Still new methods are under development, e.g. positron annihilation [8] and free solution capillary electrophoresis.[9] The spectroscopic methods are based on the fact that the physicochemical properties of a probe molecule change upon complexation due to environmental effects or to the formation of hydrogen bridges.

A very convenient method which is described and used in this chapter is fluorescence spectroscopy. If a substrate is included in the CD-cavity, it will be situated in a more apolar environment as compared to the bulk aqueous solution and, in addition, the rotational freedom of the probe will be restricted. Also the amount of shielding from the aqueous solution and from quenching molecules like oxygen, is influenced by the complexation. All these environmental effects are reflected in a change in fluorescence intensity or in a change of the emission wavelength and this makes fluorescence spectroscopy very suitable to study the formation of supramolecular complexes with cyclodextrins.

4.2 Determination of binding constants

Because the change in fluorescence intensity of a probe depends on the amount of CD complex formed, it is possible to calculate the binding constants from a titration experiment. The titration curve can be fitted using a commercially available computer program. Such a programme requires an algorithm describing the fluorescence intensity as a function of the concentration of the added host molecules. The relevant equations are derived below.

For a 1:1 complex between a host (H) and a guest molecule (G) the following equilibrium is present:

$$H + G \rightleftharpoons HG \qquad (1)$$

The binding constant (K_b) is defined as:

$$K_b = \frac{[HG]}{[H] \,.\, [G]} \qquad (2)$$

If the total amount of guest molecules ($[G]_0$) is kept constant during the titration, the concentration of unbound host [H] and guest [G] can be written as:

$$[G] = [G]_0 - [HG] \qquad (3)$$

$$[H] = [H]_t - [HG] \qquad (4)$$

In formula (4) the total amount of host molecules is represented by $[H]_t$. Substitution of (3) and (4) into equation (2) gives:

$$K_b = \frac{[HG]}{([H]_t-[HG]) \,.\, ([G]_0-[HG])} \qquad (5)$$

This can be rewritten as:

$$[HG]^2 - [HG]([H]_t + [G]_0 + K_b^{-1}) + [H]_t \,.\, [G]_0 = 0 \qquad (6)$$

From equation (6) the concentration of the complex can be calculated as follows:

$$[HG] = \frac{([H]_t + [G]_0 + K_b^{-1}) - \{([H]_t + [G]_0 + K_b^{-1})^2 - 4 \,.\, [H]_t \,.\, [G]_0\}^{1/2}}{2} \qquad (7)$$

The right hand of this formula only contains known concentrations which means that with a given K_b the concentration of complex is also known The observed fluorescence intensity (I) is the sum of the fluorescence of the free and the bound guest molecules

$$I = \phi_{HG} . [HG] + \phi_G . [G] \quad (8)$$

in which ϕ_{HG} and ϕ_G are the quantum yields for the complex and the free guest, respectively Using equation (3) to rewrite equation (8) gives

$$I = \phi_G . [G]_0 + (\phi_{HG} - \phi_G) [HG] \quad (9)$$

This formula can be seen simplified as

$$I = I_0 + C . [HG] \quad (10)$$

In formula (10) I_0 is the fluorescence of the sample before the addition of any host molecules and C is a constant representing the relative fluorescence quantum yield difference between the bound and the unbound guest From this formula it can be seen that the fluorescence depends on the amount of complex present The concentration [HG] can be described in terms of measurable quantities with equation (7) From the fluorescence intensities at various concentrations of host $[H]_t$ the values for C and K_b can be calculated in an iterative process using a curve fitting program, e g Kaleidagraph 2 0 from Abelbeck Software

In many publications also the well-known Benesi-Hildebrand (BH)-procedure [10] is used for the determination of binding constants In this procedure a double reciprocal plot is made of the fluorescence intensity and the concentration of the host, which gives a straight line for a 1 1 complex From the slope and intercept the binding constant can be obtained We have also used this method and found that in most cases the values match, within experimental error, with the values obtained using the curve fitting method It is important to note that in the BH-approach small errors in the measured values at low host concentration influence the binding constant severely On the other hand, with a curve fitting method, small errors at high host concentrations have a strong influence on the binding constant derived from the data In this chapter all the reported binding constants were determined using the fitting program

4.3 Binding of ditopic guest molecules in cyclodextrin dimers

The encapsulation of a ditopic guest molecule by two separate CD molecules in solution takes place in two steps as is shown for 6-*p*-toluidino-2-naphthalenesulphonate (**1**, TNS) in Figure 4.1

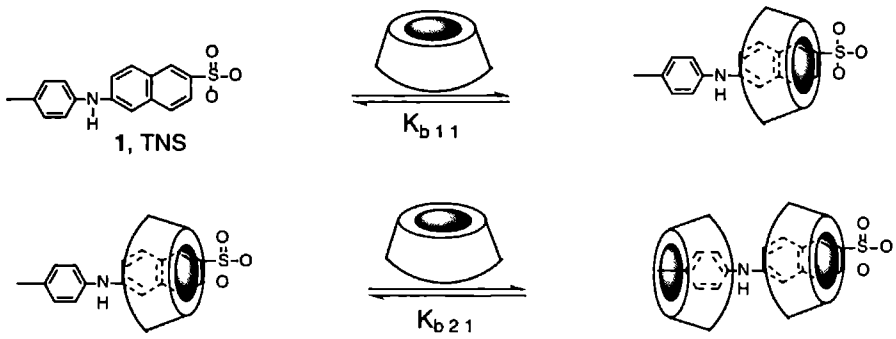


Figure 4.1 *Schematical representation of the complexation of TNS by β-CD*

The first CD binds the substrate with a binding constant $K_{b\,1\,1}$, while the second CD binds with a smaller binding constant $K_{b\,2\,1}$. For statistical reasons $K_{b\,2\,1}$ should be at least four times smaller than $K_{b\,1\,1}$ if a ditopic substrate is used [11]. In the case of TNS several articles have appeared reporting that the binding constant of the second CD is 7-200 times smaller than that of the first CD [12,13,14]. Although the ratio ($K_{b\,1\,1}$ / $K_{b\,2\,1}$) varies greatly (probably due to the large error in the determination of $K_{b\,2\,1}$), it has a tendency to be larger than 4. The lower affinity in the case of the second encapsulation is probably caused by steric hindrance between the first and the second CD-molecule. Also the energy gain due to complexation of the toluene part of TNS is probably lower than the energy gain as a result of the inclusion of the naphthyl part [15]. For the complexation of TNS in β-CD measured by fluorescence spectroscopy, Bright et al. [16] have published values for $K_{b\,1\,1}$ of 1980 (±84) M^{-1} and for $K_{b\,2\,1}$ of 600 (±95) M^{-1}. Within experimental error these numbers match the statistical factor of 4 which would indicate that there is no steric hindrance between the CDs in the second binding step. This result is strange since the experimental conditions and the method used by Bright et al. to determine the binding constants are similar to those used in the other reports. We, therefore, decided to repeat these experiments. The results will be described below (Section 4.5).

If the two CDs are covalently linked they can show a cooperative binding (or chelate) effect in the encapsulation of TNS. This is reflected in a shift of the maximum emission wavelength of

TNS in combination with an increase of the binding constant.[17] The chelate effect can be explained by the fact that the loss in translational entropy, which occurs twice on binding of two free CD moieties, only has to be paid once when the CDs are connected to each other, because the entropy loss of the second CD has already been paid for in the synthesis of the dimer. This classical explanation[18] of the chelate effect in entropy terms has recently been questioned by Zhang and Breslow.[19] They studied the complex formation of ditopic guests with β-CD and with CD-dimers using microcalorimetry. From the thermodynamic parameters it was concluded that the chelate effect found for CD-dimers is due to an increased binding enthalpy. No explanation for the improved enthalpy of binding was given. The influence of the linking spacer was considered to be of no importance. In our opinion this part of the molecule, however, can give an extra hydrophobic interaction with the guest molecule resulting in a more efficient binding process. From the work of Zhang and Breslow it is clear that an explanation of the binding of ditopic guests by CD-dimers in terms of entropy alone is not sufficient.

In this chapter the binding of several substrates by cyclodextrin dimers will be reported and evaluated in terms of cooperative binding. Another interesting subject that will be discussed is the shape complimentarity between the substrate and the host molecule. For example, the asymmetrical fluorescent probe TNS is known to form both 1:1 and 2:1 (host:guest) complexes with β-CD and only 1:1 complexes with α-CD because the naphthyl part of the probe does not fit into the α-CD unit.[13] It should be possible, therefore, to bind TNS in a site-specific way using CD hetero-dimers consisting of a small α-CD and a larger β-CD-unit. This is schematically drawn in Figure 4.2.b. To investigate this site-specific binding in more detail we synthesised two other probes as will be described in the next section.

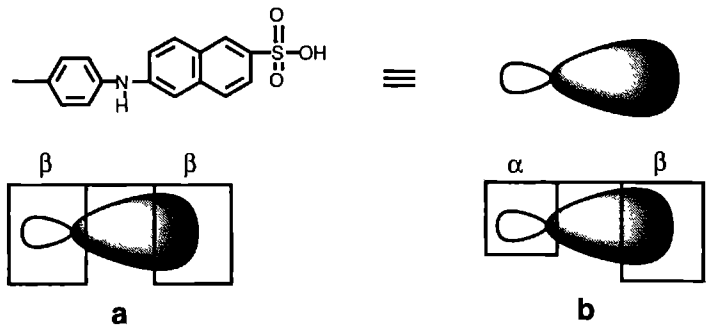
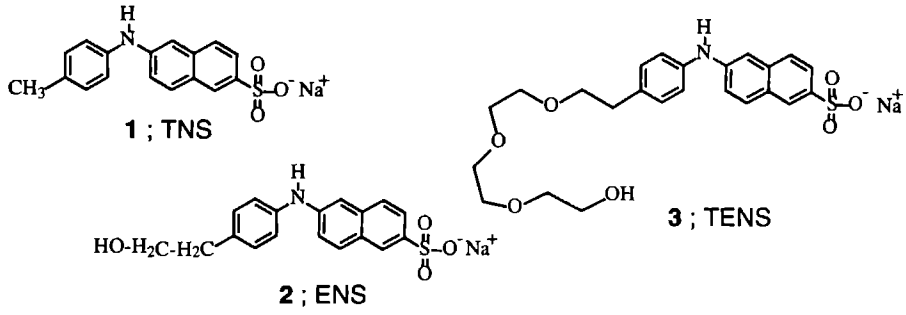


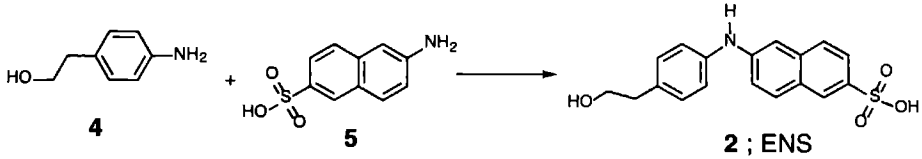
Figure 4.2 *Non site-specific (a) and site-specific (b) binding of TNS in cyclodextrin dimers.*

4.4 Synthesis of fluorescent probes

Since the binding constant of the complex between α-CD and TNS is only 18 M^{-1},[13] cooperative binding effects in the hetero-dimers might be difficult to observe. We decided, therefore, to synthesise two new TNS analogues, which are 2-(*p*-(2'-hydroxyethyl)anilino)-6-naphthalene sulphonate (**2**) and 2-(*p*-(3',6',9'-trioxa-11'-hydroxyundecane)anilino)-6-naphthalene sulphonate (**3**) for which we propose the names ENS and TENS, respectively. In these probes, the methyl group at the para position of the phenyl-ring of TNS is replaced by an hydroxyethyl- or a 3,6,9-trioxa-11-hydroxyundecyl group.

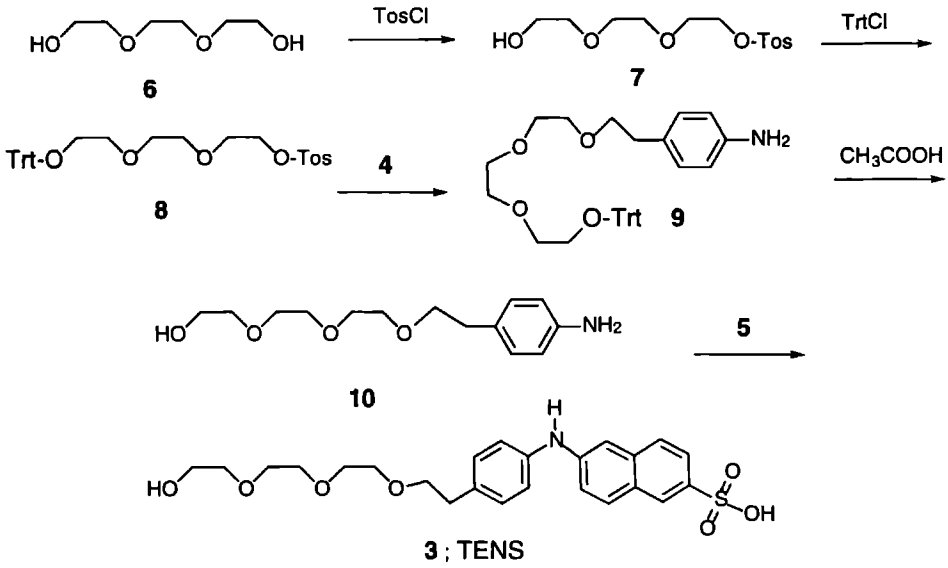


It is known that polyethyleneglycol can form inclusion complexes with α-CD and not with β-CD.[20] In these complexes, which involve many CD rings, each α-CD molecule encapsulates approximately a triethyleneglycol unit.[21] We, therefore, expected that the complex with our CD hetero-dimer would be stabilised if the side chain of the guest would be at least three ethyleneoxy units long.



Scheme 4.1

The synthesis of the fluorescent probe ENS was carried out by a Bucherer reaction, following the procedure described by Cory et al. (Scheme 4.1).[22] Reaction of 6-amino-2-naphthalenesulphonate **5** with 2-(4-aminophenyl)ethylalcohol **4** in the presence of sodium bisulphite gave, ENS in 53% yield after recrystallisation. The synthesis of the probe TENS involved more reaction steps (Scheme 4.2).



Scheme 4.2

It started with the monotosylation of triethyleneglycol using NaH as a base giving compound **7** in 80% yield. It was not possible to obtain synthon **10** in a direct reaction between **7** and compound **4**. The hydroxyl group of compound **7**, therefore, was first protected with a triphenylmethyl (Trt) group. The protected compound **8** reacted readily with the alkoxy anion of compound **4** to give **9** in 70 % yield. To make the latter compound soluble in water, as required for the next reaction, it was deprotected using acetic acid to give compound **10**, which was used in the final Bucherer reaction with **5**. The yield of TENS was only 7%. No effort was made to improve this yield.

4.5 Binding studies with TNS-derivatives and cyclodextrin dimers

As was described in Section 4.3 the inclusion of anilinonaphthalenesulphonates in CD-derivatives can be studied by fluorescence spectroscopy. A titration in which the fluorescence intensity is monitored as a function of the concentration of host molecules at a fixed concentration of probe molecules allows the calculation of the binding constant. A typical titration curve of TNS with β-CD is shown in Figure 4.3.

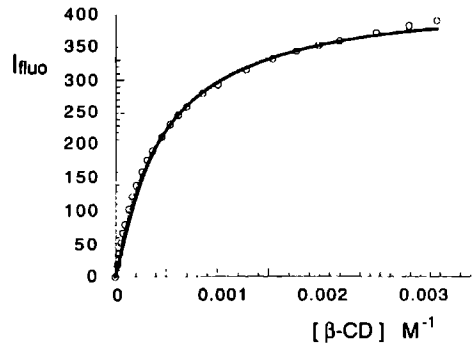
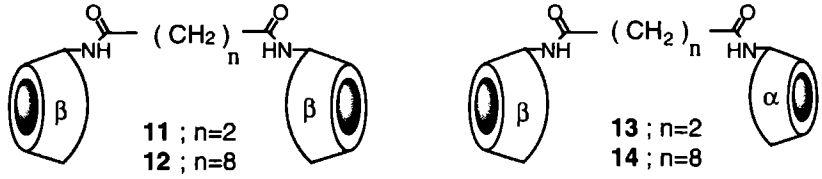


Figure 4.3 *Titration curve of TNS with β–cyclodextrin (λ_{ex}=322 nm, λ_{em}=459 nm)*

In our experiments, titrations were performed with the three fluorescent probes TNS, ENS and TENS using the homo-dimers **11** and **12** and the hetero-dimers **13** and **14** which were described in Chapter 2 (for experimental details on the titrations see Experimental section).



The calculated binding constants (K_b's) are summarised in Table 4.1. The K_b values for α-CD and β-CD were also determined for comparison. As expected, all probes are bound more tightly to the symmetrical β-CD-dimers, **11** and **12**, than to monomeric β-CD, which indicates a cooperative binding process. On going from TNS via ENS to TENS the binding constants decrease for CD-dimers **11** and **12**. This result can be explained from CPK-models of the dimers and the probes. The hydrophobic aromatic region of TNS is approximately 14 Å long while the length of the hydrophobic region of the CD-dimers is larger than 16 Å (value for two CD rings in close proximity). This means that the probes ENS and TENS, in contrast to TNS, will use their hydroxyethyl and ethyleneglycol chains to fill the CD-cavities. As a result the energy gain upon complexation, due to hydrophobic interactions, will be smaller for these probes than for TNS, which is reflected in the decrease of their binding constants. For the hetero-dimers **13** and **14**, the opposite effect is observed. Despite the fact that the probes become more hydrophilic, the binding constants increase on going from TNS via ENS to TENS. This is due to the larger length of the side chain in ENS and TENS as compared to TNS. The first two probes can form a more stable complex with the α-CD part of the dimer

Table 4.1 *Binding constants (in M^{-1}) for several combinations of probes and hosts* [a]

Host molecule	TNS	ENS	TENS
α-CD	25 ± 10	25 ± 10	30 ± 10
β-CD (1 1)	2 100	2 400	2 500
(2 1)[b]	200 ± 100	- [c]	- [c]
11	10 500	8 000	6 000
12	6 700	4 100	3 800
13	2 800	3 000	4 000
14	600	1 000	1 400

[a] The estimated error in the binding constants is 5 % unless indicated otherwise [b] Determined using a previously reported procedure see refs 13, 14 [c] Not determined

than TNS because their side chains fit in more tightly This favourable fit compensates for the negative effect of binding a hydrophilic chain in an apolar cavity The binding profiles observed for hetero-dimers **13** and **14** in combination with the binding profiles of the symmetrical dimers **11** and **12** are a very strong indication that site-specific guest binding occurs in the former set of host molecules

Table 4 1 reveals that the CD-dimers with short linkers (**11** and **13**) display higher binding constants than the dimers with long spacers (**12** and **14**) This is consistent with the observations of Petter et al [23] who suggested that the longer the linking spacer between two CDs is, the more entropy must be quenched to form a highly ordered inclusion complex Our NMR studies on CD-dimers described in Chapter 2, however, may offer another, more likely explanation of the results From our studies it was concluded that the long alkyl spacers of dimers **12** and **14** are encapsulated by one of the two CD-moieties It is necessary to remove these spacers from the CD-cavities before the substrate can be bound In this process the spacer is forced to go into the aqueous solution which is energetically unfavourable This is reflected in the lower binding constants Self-encapsulation of the spacer may also have played a role in the binding processes studied by Petter et al [23] and may have contributed to the lower binding constants found for some of their dimers

The position of the emission maximum (λ_{max}) in the fluorescence spectra of the complexes of the anilinonaphthalenesulphonates with the CD-derivatives can provide information as to what extent the probe is shielded from the aqueous solution The observed values of λ_{max} are summarised in Table 4 2 These values can also be used to obtain information on the binding

Table 4.2 *Position of the emission maximum (nm) in the fluorescence spectra of complexes between CDs and probe molecules* [a]

Host-molecule	TNS	ENS	TENS
α-CD	453	442	440
β-CD (1:1)	459	453	451
(2:1)	446	- [b]	- [b]
11	440	434	433
12	436	430	430
13	453	450	447
14	453	450	448

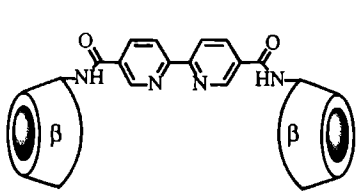
[a] Excitation wavelengths are 322, 318, and 319 nm for TNS, ENS and TENS, respectively, [probe] = 1 x 10^{-5} M, [CD] = 1 x 10^{-3} M, [CD-dimer] = 1 x 10^{-4} M.
[b] Not determined.

stoichiometry of the complexes. For example, if TNS is encapsulated by only one β-CD ring, the λ_{max} amounts to 459 nm, whereas this value shifts to 446 nm if the probe is encapsulated by two β-CD-moieties.[14] The values found for the complexes between TNS and dimers **11** and **12** (Table 4.2) indicate that TNS is encapsulated by both CD-cavities and that the shielding effect of a dimer is even more efficient than the shielding of two non-covalently linked CD molecules. As for ENS and TENS similar λ_{max} values are observed as for TNS it can be concluded that these two probes are also encapsulated by two cavities. With dimer **12** a more efficient shielding of the probes is achieved than with **11**. This additional shielding is probably caused by the fact that the apolar spacer is larger in the former CD-dimer than in the latter. In the case of the hetero-dimers the λ_{max} values are close to the values measured for the 1:1 complexes of β-CD. Apparently, no strong shielding effect results from the α-CD unit in the hetero-dimer. It is clear from Table 4.2 that the encapsulation of a probe by free α-CD results in a larger decrease in the value of λ_{max} than encapsulation by a free β-CD. This decrease is not clearly observed in the case of the complexes with the hetero-dimers, indicating that the shielding contribution of α-CD in the hetero-dimers is different from the shielding by free α-CD. A possible explanation for this result might be that the λ_{max} value depends on the binding geometry of the probe-α-CD complex.[24] For example, the encapsulation of TNS via the primary side of α-CD might result in a more efficient shielding (a lower value of λ_{max}) than is possible for a complex in which the toluene unit is encapsulated via the secondary side. In the complexes with **13** and **14** the probe can only enter via the secondary side of the α-CD ring, which may explain the observed effect.

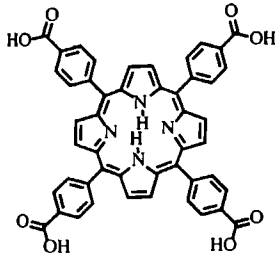
4.6 Binding studies with porphyrins

As discussed in Chapter 3 the combination of CD-dimers and porphyrins may be of interest for the development of new catalytic systems. In this chapter the complexation of two porphyrin derivatives in different CD-dimers will be described This study was undertaken to obtain more insight in the binding geometry of the complexes that were used in the catalytic system described in Chapter 3. Some examples of complexes between porphyrins and (methylated) cyclodextrins have been reported in the literature and several 2:1 cyclodextrins-porphyrin complexes have been synthesised and used to mimic haem-containing proteins, e g. Cytochrome P-450 [25,26,27] Also a 4:1 β-CD-zinc tetrakis(4-sulphonatophenyl)porphyrin (ZnTsPP) complex has been described.[28] The formation of this 4:1 complex was proposed on the basis of kinetic studies and spectroscopic changes.[29] Since no binding constants were determined and only very few data points were collected, we feel that the proposed structure should be considered as being a tentative one. For steric reasons it is not very likely that four CD-units will encapsulate one porphyrin molecule

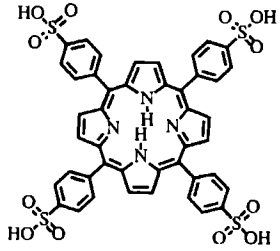
In the catalytic experiments described in the previous chapter we used porphyrins that contain a manganese(III) ion as the active metal centre Since these manganese porphyrins do not give any fluorescence signals, the metal free tetrakis(4-benzoic acid)porphyrin (TcPP, **16**) and tetrakis(4-sulphonatophenyl)porphyrin (TsPP, **17**) were used in the present studies. To avoid spectral overlap with the excitation wavelength of the porphyrin we used the Rh-free CD-dimer **15** instead of the Rh complex that was tested in Chapter 3 for our studies. For comparison the two symmetrical CD-dimers **11** and **12** were also investigated



15



16; TcPP



17; TsPP

Porphyrins are known to aggregate in aqueous solution.[30,31,32] We decided therefore to first investigate the aggregation behaviour of TcPP and TsPP by making use of the fact that aggregation leads to quenching of the fluorescence of these molecules [32] In Figure 4.4 the fluorescence intensities of the porphyrins are plotted as a function of their concentrations.

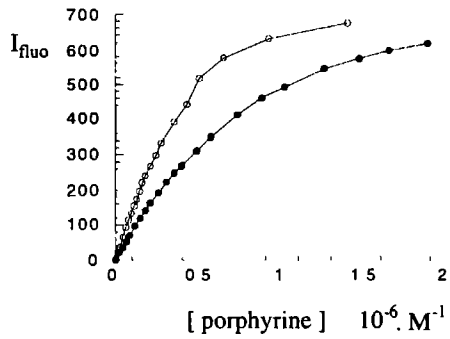


Figure 4.4 *Fluorescence intensities of TcPP (open circles) and TsPP (closed circles) in aqueous solution as a function of their concentrations.*

Above approximately 2.10^{-6} M plateau values are reached and at higher concentrations (>$2.5.10^{-6}$ M) also inner filter effects[33] were observed. As can be seen from the plot, binding studies should be performed below a concentration of 5.10^{-7} M, as in this range the effect of self-quenching is limited. Binding studies were carried out, therefore, at very low concentrations (2.10^{-7} M) of porphyrins to ascertain accurate results.

In a first series of experiments the complexation of TcPP in β-CD was studied. From the decrease of the fluorescence intensity as a function of the β-CD concentration we calculated a binding constant of K_b = 1700 M^{-1} (see Experimental for details). This *decrease* is in striking contrast with the observed *increase* in fluorescence intensity reported in the literature by Zhou and Luong.[34] On the basis of UV-vis measurements and ^{13}C-NMR-spectra they proposed that TcPP is situated as a lid over the primary side of β-cyclodextrin, see Figure 4.5.a.

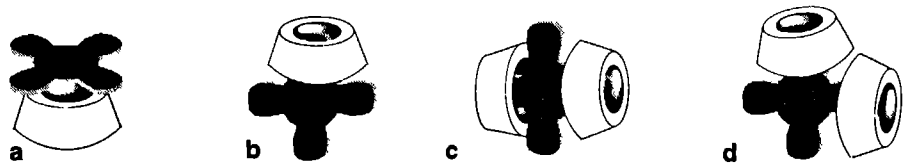


Figure 4.5 *Possible structures of complexes between porphyrins and cyclodextrins.*

Since the porphyrin concentration used by the authors (5.10^{-6} M) is in the aggregation range of TcPP, it is likely that they have observed the breakdown of porphyrin aggregates rather than the formation of the complex depicted in Figure 4.5.a. This deaggregation would also explain the

changes that were observed by Zhou and Luong in the NMR-spectrum of TcPP when the cyclodextrin derivative was added to the porphyrin. The binding constant they reported for the β-CD-TcPP complex is $K_b = 7.10^4\ M^{-1}$. This value and other data reported in their article are probably incorrect. We propose that the porphyrin TcPP is located *in* the CD cavity (Figure 4.5.b) and not situated *over* the cavity (Figure 4.5.a). The former type of complex would be in agreement with NOESY-spectra of CD-porphyrins complexes reported by Lawrence et al. [27] and with ROESY-spectra described by Ribó et al..[24] These studies indicate that when a porphyrin is encapsulated by two (2-6-permethylated) CDs, the latter molecules are located on opposite phenyl groups of the porphyrin, as depicted in Figure 4.5.c.

In a second series of experiments we studied the complex formation between the CD-dimers **11** and **12** and the water-soluble porphyrins TcPP and TsPP. In this case complexation also resulted in a decrease of the fluorescence intensities of the porphyrins. From the titration curves (Figure 4.6.a) the binding constants could be obtained in the same way as described for the TNS complexes (Section 4.2). The results are presented in Table 4.3.

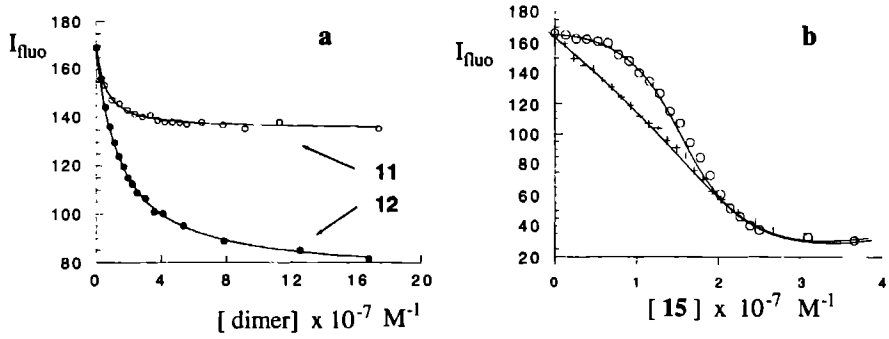


Figure 4.6 *Plots of the fluorescence intensity of TsPP as a function of (a) the concentration of dimers **11** and **12** and (b) the concentration of dimer **15** with (+) and without Zn^{2+} (0.5 equiv.) present (o).*

As compared to monomeric β-CD, the CD-dimers display very high binding constants, which indicates a strong cooperative effect between the two CD-cavities. A lower binding constant is observed if the alkyl spacer between the two CD rings is longer, compare **11** and **12**. This difference in binding affinity can be explained by the self-encapsulation of the linking spacer, which occurs in dimer **12** but not in dimer **11** (see also Section 4.3).

Table 4.3 *Binding constants (M^{-1}) of complexes between cyclodextrins and the porphyrins TcPP and TsPP.*

Host molecule	Guest molecule	
	TcPP [a]	TsPP [a]
β-cyclodextrin	1700	1400
11	19×10^5	8×10^5
12	9×10^5	4×10^5

[a] Estimated error in binding constants is 10%.

To investigate the binding geometries of the CD-dimer-porphyrin complexes, we recorded ^{1}H-NMR spectra of the complexes between TsPP and dimers **11** and **12** (spectra not shown). In the case of a porphyrin-dimer complex two complexation geometries are possible, i.e. an anti geometry and a syn geometry (see Figures 4.5.c and 4.5.d, respectively). For the pyrrole ring protons of the porphyrin four signals, two singlets and two doublets, can be expected if the complex has the syn geometry and two doublets if the complex has the anti geometry.[35] In the ^{1}H-NMR spectra of the TsPP-**11** complex only one broad signal was visible for the porphyrin ring protons at room temperature, but four broad signals (δ = 9.17, 8.92, 8.82, and 8.61 ppm) were observed at -10 °C (25 % (v/v) CD_3OD was added to prevent freezing of the solution, the influence of this cosolvent on binding constants is probably small [37]). This result suggests that dimer **11** forms a syn complex. In the case of the TsPP-**12** complex the ^{1}H-NMR spectra at -10 °C revealed the same four signals (δ = 9.17, 8.90, 8.83, and 8.62 ppm) as well as two extra peaks at δ = 8.95 and 8.51 ppm, suggesting the presence of a mixture of syn and anti complexes. From the intensity of the signals the ratio of the syn/anti conformers could be calculated to be 2:1. If the geometry of the porphyrin-dimer is anti the protons of the methylene spacer are located in the shielding zone of the porphyrin ring and therefore should display a large upfield shift. For the TsPP-**11** complex such a shift was not observed. The TsPP-**12** complex, however, displayed several broad signals for the CH_2-protons in the region of 1 to -2 ppm. These results together with the observed patterns of the signals of the porphyrin ring protons strongly indicate that syn complexes are present in the case of dimer **11** and a mixture of syn and anti complexes are present in the case of dimer **12**. In Figure 4.9 the computer generated drawings of these complexes are shown.

The titration curve of the bipyridine-containing dimer **15** with TsPP is shown in Figure 4.6.b. This curve clearly is different from those observed for dimers **11** and **12** and could not be fitted assuming a simple 1:1 complex formation. We propose that the following binding processes take place:

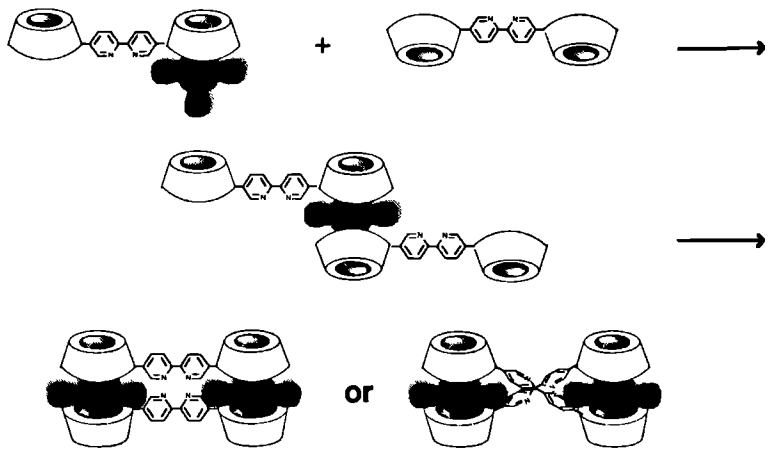


Figure 4.7 *Complexation process between cyclodextrin dimer **15** and porphyrin TsPP*

First a 1:1 complex between dimer **15** and TsPP is formed in which only one CD-cavity is involved due to the rigidity of the spacer. When more CD-dimer is added a 2:1 (dimer:TsPP) complex can be generated. At this point the two remaining cavities behave like a cyclodextrin dimer with a "flexible spacer" and therefore a second porphyrin molecule can be easily bound. These processes would explain the sigmoidal shape of the binding curve. For the 2:2 complex two conformations are possible, one in which the bipyridyl units are oriented in such a way that a metal centre with a tetrahedral geometry can be complexed and one in which these units have a parallel orientation, allowing a metal centre with an octahedral or square-planar coordination geometry to be bound. To study the influence of a coordinating metal ion on the complexation behaviour of TsPP with dimer **15**, a titration curve was also recorded in the presence of 0.5 equivalent of Zn(II) perchlorate or Rh(III) trichloride. The addition of these metal ions in a metal free buffer (0.01 M Tris, pH 7.0) changed the shape of the curve significantly but did not influence the ratio at which the plateau value occurred (see Figure 4.6.b). Apparently, the complex now is easier formed because the 2:2 geometry can be stabilised by coordination of the metal ion. Although the curves for Rh and Zn were very similar, the complexes are probably different because Zn(II) will prefer to be tetrahedrally coordinated by the bipyridines whereas the Rh(III) centre will prefer an octahedral arrangement of the two bipyridines with two chlorine anions as axial ligands.[36]

A direct determination of the binding constant for the 2:2 complex was not possible but an estimation can be made from Figure 4.6.b : $K_b > 5 \times 10^7\ M^{-1}$. In order to get information on their relative molecular weights, the complexes were applied to gel chromatography. The normalised chromatograms are presented in Figure 4.8. This figure reveals that the elution volume of a dansyl-appended β-CD (molecular weight approx.1400, see Chapter 5) is much larger than the elution volume of free dimer **15** as can be expected from their relative molecular

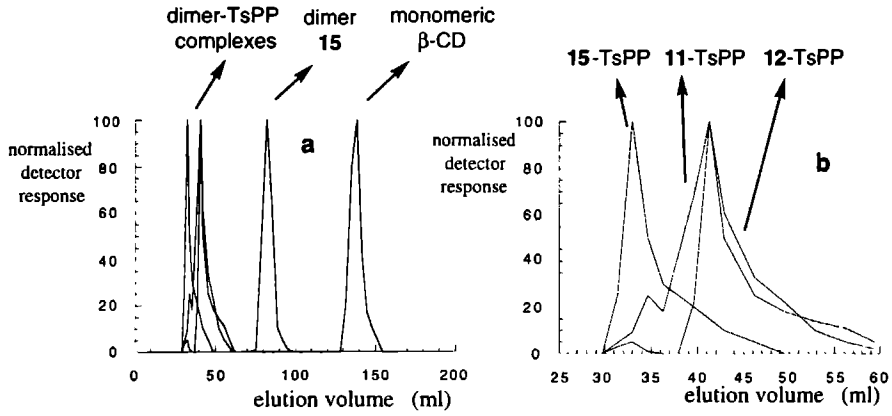


Figure 4.8 *Gel chromatograms of complexes between CD-dimers and TsPP (a). An enlargement is presented in (b). Fractogel column, bed volume 200 ml, eluent water, flow rate 13.2 ml.h^{-1}.*

weights. All complexes between dimers and TsPP show smaller elution volumes than **15** itself, which clearly indicates that the complexes have a higher mass. After lyophilisation of the fractions that contained the complexes, the presence of both porphyrin and cyclodextrin was confirmed by ^{1}H-NMR. From the enlargement in Figure 4.8.b it is obvious that the molecular weight of the TsPP-**15** complex is larger than that of the complexes between TsPP and **11** or **12**. This must be due to the formation of a 2:2 complex and the formation of 1:1 complexes, respectively. From Figure 4.8.b it can be concluded that small fractions of the complexes between TsPP and dimers **11** and **12** also have a 2:2 geometry. These results show that gel chromatography is a powerful tool for the characterisation of supramolecular complexes. This technique is probably limited to complexes that have high binding constants.

Time of flight mass spectrometer measurements, which were carried out to get more evidence for the proposed structures, gave no information about the molecular weights of the complexes between porphyrins and cyclodextrins due to significant interference of sodium ions. These ions were present since all complexation studies were performed starting from the tetrasodium salt of TsPP.

Preliminary ^{1}H NMR experiments were carried out in order to verify the proposed 2:2 structure between TsPP and **15**. 400 MHz spectra of this complex in D_2O showed, in particular for the porphyrin and bipyridine resonances, significant line broadening indicative of an exchanging system which prohibited spectral assignment. Upon addition of 0.5 equivalent of $ZnCl_2$ line sharpening occurred suggesting the presence of a more rigid complex. COSY and NOESY experiments (200 MHz) on this complex were carried out which enabled the assignment of the porphyrin and bipyridine resonances. For the porphyrin four individual pyrrole resonances were observed at δ = 9.21, 8.98, 8.82, and 8.34 ppm while for the phenyl protons three

different AB quartets were observed: δ = 7.83, 8.21; 7.98, 8.18 and 8.16, 8.42 ppm in a ratio of 1:1:2, respectively. These signals can not be explained assuming a 1:1 complex between the porphyrin and dimer **15**. The spectra, however, can be interpreted assuming a 2:2 complex with two sets of two porphyrin phenyl groups bound within the cyclodextrins in an anti manner (signals at 8.16 and 8.42 ppm). The other phenyl groups appear as two different signals probably because two of them are pointing toward the centre of the complex, thereby being located close to the positively charged Zn(II) ion, and two other ones are pointing out of the complex. Since a full assignment of all protons has not been made yet, further experiments must be carried out to unequivocally prove the 2:2 complex structure which is shown as a computer generated drawing in Figure 4.9.

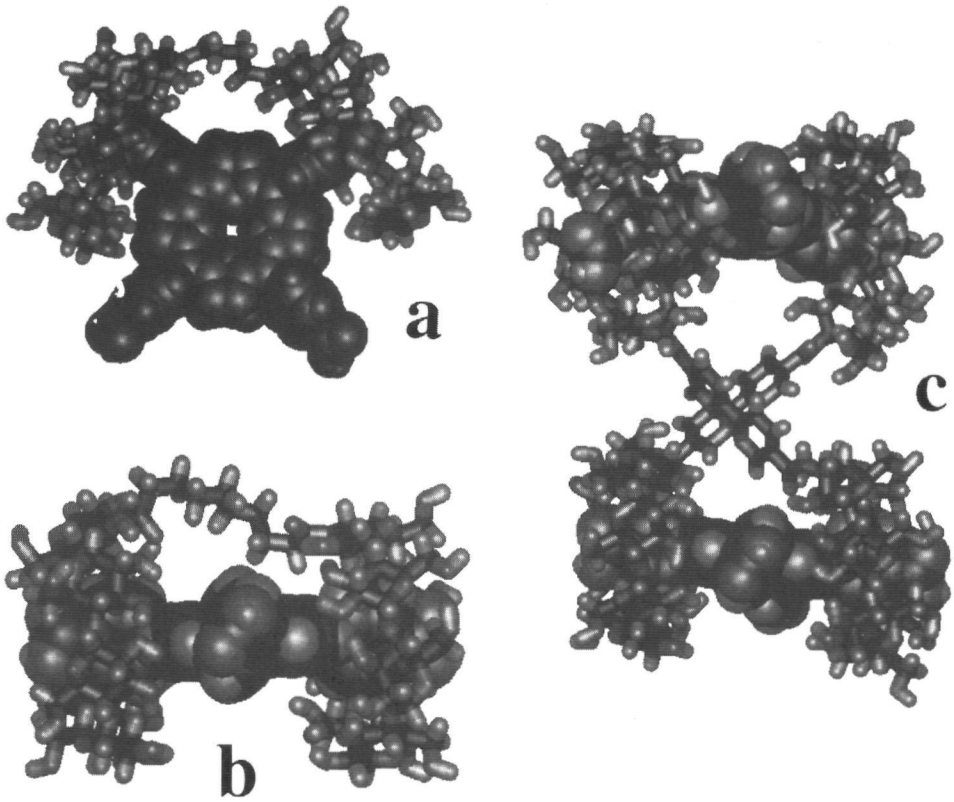


Figure 4.9 *Computer generated structures of cyclodextrin-porphyrin complexes, porphyrins are represented as space filling models whereas cyclodextrin dimers are represented as stick and balls for reasons of clarity; (a) syn-complex between TsPP and dimer **11**; (b) anti-complex between TsPP and dimer **12**; (c) 2:2 complex between TsPP and dimer **15**.*

4.7 Conclusions

In this chapter we have shown that cooperative binding of ditopic guest molecules can be achieved in cyclodextrin dimers that are linked via their secondary sides. By using cyclodextrin hetero-dimers it is possible to bind anilinonaphthalenesulphonates in a site-specific way. The complexation geometry of two porphyrins bound in CD-dimers has been investigated by fluorescence and ^{1}H-NMR spectroscopy. The results indicate that a small, flexible spacer between the CDs leads to syn complexes, whereas a large spacer yields a mixture of syn and anti complexes. If a rigid spacer is used, viz. a bipyridine-unit, a 2:2 complex is formed. The existence of such a complex could be supported by gel chromatography and preliminary NMR studies.

The results presented in this chapter open the way to new catalytic systems. For example, regioselective and stereoselective reactions may be achieved by using a CD hetero-dimer, which can bind a substrate in a regioselective way. CD-dimers can also be used as mimics of the protein coating of natural haem groups, and may be useful to diminish the effect of porphyrin aggregation, which will lead to more effective catalysts or better enzyme mimics.

4.8 Experimental

Fluorescence measurements: Fluorescence measurements were performed with a Perkin-Elmer luminescence spectrometer LS50B. A 1.00 cm quartz cuvette (4 ml) was used, which was placed in a thermostated (25.0 ± 0.1 ^{0}C) cuvette holder. All measurements were carried out using a 0.1 M buffer (pH 7.0) of KH_2PO_4 in distilled or demineralised water. The concentration of the fluorophore was kept constant in every experiment by using stock solutions of known concentrations. Typical concentrations were 1 x 10^{-5} M for the TNS-derivatives and 2 x 10^{-7} M for the porphyrins. A known amount of CD(-dimer) was added to a portion of these stock solutions yielding a solution with known concentrations of CD and fluorophore. The concentration of CD(-dimer) that was used, depended on the binding constant of the complex and was chosen in such a way that the titration yielded at least 10 data points in the region where 20-80% of complex is formed. This region allows the most accurate determination of binding constants.[38] The excitation wavelengths that were used, are (nm): TNS, 322; ENS, 318; TENS, 319; TsPP, 413 and TcPP, 415. The excitation slits were 2.5 nm and the emission slits were between 2.5-10 nm depending on the fluorescence intensity of the probe under investigation. No oxygen quenching of the fluorescence was observed. Significant photodecomposition of the porphyrins occurred if the excitation slits were >2.5 nm and if the scan speeds were <60 nm.min^{-1}. A scanning speed of 120 nm.min^{-1} over a region of only 20 nm therefore was used in the case of the porphyrins studied. The titrations were carried out by starting with a solution of the probe compound (2000 µl) and adding small portions of the CD-stock solution (5-1000 µl). After every addition a spectrum was recorded which was stored in a computer. By substracting the first from the last spectrum a difference spectrum was obtained, which revealed at what wavelength the maximum change in the fluorescence intensity had

occurred Subsequently, the fluorescence intensity at this wavelength was determined in the stored spectra The intensities were plotted as a function of the CD(-dimer) concentration These data were fitted assuming a 1 1 complex, as described in Section 4 1 All measurements were performed in duplo

Gel chromatography Gel chromatography was performed using standard procedures for column packing A column (length 30 cm, bed volume 200 ml) was filled with Fractogel (TSK HW-40 (F), Merck) The flow rate was 13 2 ml per h The exclusion limit of the column for carbohydrates was 7000 g mol ^{1} as quoted by the manufacturer Compounds were detected with an UV-detector (LKB Uvicord S 2138) at 278 nm The fractions that gave a signal on the detector were checked after lyophilisation for the presence of CD-derivatives and porphyrins by ^{1}H-NMR (90 MHz)

NMR-spectra of complexes: ^{1}H-NMR-spectra (400 MHz) were recorded in a mixture of D_2O and CD_3OD (75/25, v/v) [porphyrin] = 1 5 x 10 ^{3} M, [cyclodextrin dimer] = 3 - 4 5 x 10 ^{3} M Before the acquisition, the sample was presaturated to reduce the HDO signal

Synthesis: For general experimental details of the synthetic procedures see Section 2 6 Chemicals were used as received from the manufacturer, except tosyl chloride which was first recrystallised from n-hexane

2-(*p*-(2'-Hydroxyethyl)anilino)-6-naphthalenesulphonate (ENS, 2)

2-Amino-6-naphthalenesulphonate (**5**) (1 75 g, 7 88 mmol), 291 mg of NaOH (7 28 mmol) and 17 g of $NaHSO_3$ were dissolved in 50 ml of water After the addition of 2 0 g (14 6 mmol) of 2-(4-aminophenyl)ethylalcohol (**4**) the reaction mixture was refluxed for 72 h On cooling a precipitate was formed which was removed by filtration After recrystallisation (twice) from water the resulting slightly yellow solid was collected and dried *in vacuo* Yield 1 40 g (53%) Mp >350 °C FAB-MS (m/e) 365 (M + 1) ^{1}H-NMR (D_2O/DMSO-d6, 10/1, v/v, 90 MHz) 8 25 (s,1H), 8 0 and 7 9 (d,1H), 7 7 (m,2H), 7 45 (m,2H), 7 25 (m,4H,phenyl), 3 8 (t,2H,CH_2-OH), 2 8 (t,2H,CH_2-Ar) Anal Calcd for $C_{18}H_{16}NSO_4Na$ C, 59 17, H, 4 41, N, 3 83 Found C, 58 95, H, 4 30, N, 3 81

8-Tosyloxy-3,6-dioxaoctanol (7)

To a suspension of 4 34 g of cleaned NaH (110 mmol) in 750 ml of THF was added 150 g of triethylene glycol **6** (100 mmol) After gas evolution had stopped, a solution of 19 06 g of tosyl chloride (100 mmol) in 25 ml of THF was added in 15 min After 18 h the reaction mixture was concentrated *in vacuo* and the residue dissolved in 250 ml of ethyl acetate The resulting solution was washed with water and brine, dried over $MgSO_4$ and concentrated to give a colourless oil According to TLC and NMR this oil was pure product **7** Yield 24 33 g (80%) EI-MS (m/e) 305 (M + 1) ^{1}H-NMR ($CDCl_3$, 100 MHz) 7 80 (d,2H,*H*-Ar), 7 35 (d,2H,*H*-Ar), 4 11 (t,2H,CH_2-O-Tos), 3 74-3 51 (m,10H,CH_2-O), 2 45 (s,3H,CH_3-Ar)

1,1,1-Triphenyl-10-tosyloxy-2,5,8-trioxadecane (8)

Compound **7** (15 g, 50 mmol) was dissolved in 300 ml of THF and 5 ml of pyridine was added Triphenylmethyl chloride (13 7 g, 50 mmol) was added and the reaction mixture was refluxed for 20 h After concentration of the reaction mixture *in vacuo*, the residue was

dissolved in ethyl acetate. The solution was washed with water and brine, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (900 g silica 60; eluent: ethyl acetate:hexane, 1:9, v/v), yielding compound **8** as a yellow oil. Yield: 10.84 g (40%). ^{1}H-NMR ($CDCl_3$,90 MHz): 7.7 (d,2H,*H*-Ar), 7.5-7.0 (m,17H,*H*-Ar), 4.1 (t,2H,C*H*$_2$-OTos), 3.7-3.5 (m,8H,C*H*$_2$-O-Tos), 3.2 (t,2H,C*H*$_2$-OTrt), 2.4 (s,3H,C*H*$_3$-Ar).

13-(4'-Aminophenyl)-1,1,1-triphenyl-2,5,8,11-tetraoxatridecane (9)

Compound **8** (2.6 g, 4.76 mmol) and 627 mg of 2-(4-aminophenyl)ethylalcohol (**4**, 0.95 equiv.) were dissolved in 60 ml of THF and 513 mg of potassium-*tert*-butoxide (0.95 equiv.) was added in one portion. After 6 h the reaction mixture was concentrated *in vacuo* and the residue dissolved in ethyl acetate. The solution was washed with water and brine, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (200 g silica 60; eluent 1% MeOH (v/v) in $CHCl_3$). This yielded compound **9** as an orange oil. Yield: 1.7 g (70%). ^{1}H-NMR ($CDCl_3$) : 7.5-7.1 (m,15H,*H*-Trt), 6.9-6.8 (d,2H,Ar-*H*), 6.5-6.4 (d,2H,Ar-*H*), 3.8-3.3 (m,12H,C*H*$_2$-O), 3.2 (t,2H,C*H*$_2$-OTrt), 2.8 (t,2H,C*H*$_2$-Ar).

11-(4'-Aminophenyl)-3,6,9-trioxaundecanol (10)

Compound **9** (805 mg, 1.37 mmol) was dissolved in 150 ml of acetic acid and kept at 40 ^{0}C for 24 h. Subsequently, 150 ml of aqueous 1 M HCl was added and the reaction mixture was washed with ethyl acetate to remove unreacted starting compound. NaOH was added to the mixture until the pH was 12. The product was extracted with ethyl acetate and the organic layers were dried over $MgSO_4$ and concentrated. After purification by column chromatography (80 g silica 60, eluent 3% MeOH in $CHCl_3$) compound **10** was obtained as an orange oil. Yield: 248 mg (59%). EI-MS (m/e): 269 (M + 1). ^{1}H-NMR ($CDCl_3$, 90 MHz) : 6.9-6.8 (d,2H,Ar-*H*), 6.5-6.4 (d,2H,Ar-*H*), 3.8-3.3 (m,14H,C*H*$_2$-O), 2.8 (t,2H,C*H*$_2$-Ar).

2-(*p*-(3',6',9'-Trioxa-11'-hydroxyundecane)anilino)-6-naphthalenesulphonate (TENS, 8)

In 5 ml of water were dissolved 98 mg (0.40 mmol) of 2-amino-6-naphthalenesulphonate (**4**) and 16 mg of NaOH (1 equiv.). This solution was added to 107 mg (1 equiv.) of compound **10**. After addition of 2.5 g of sodium bisulphite the reaction mixture was refluxed for 48 h. Hereafter, 25 ml of water was added and the aqueous solution was extracted with ethyl acetate to remove unreacted **10**. The water layer was concentrated *in vacuo* and the resulting residue was extracted by stirring with ethanol (3 x) and the combined ethanol fractions were concentrated to a small volume. This solution was poured into hexane and the resulting red precipitate was collected by centrifugation. Yield: 13.9 mg (7%). Mp >350 ^{o}C. FAB-MS (m/e): 520 (M + Na). ^{1}H-NMR (D_2O, 90 MHz) : 8.1-6.6 (m,10H,Ar-*H*), 3.8-3.1 (m,14H,C*H*$_2$-O), 2.8 (t,2H,C*H*$_2$-Ar). Anal. Calcd for $C_{24}H_{28}NSO_7Na.4H_2O$: C, 49.23; H, 6.20; N, 2.39; S, 5.47. Found (after correction for the presence of salts (approx. 10%)): C, 49.09; H, 6.04; N, 2.74; S, 5.96.

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CHAPTER 5

Dansyl Appended Cyclodextrins as Sensor Devices

5.1 Introduction on cyclodextrin based sensors

Cyclodextrins (CDs) are spectroscopically rather inert. Inclusion phenomena involving these molecules, therefore, are usually studied with the help of spectroscopically active guest molecules, which exhibit changes in absorption or fluorescence spectra when they are complexed. Upon modification with chromophoric groups, cyclodextrins become spectroscopically active. Such derivatives can be used in the case of guest molecules that can not be detected easily by spectroscopic methods. Applying CDs for the detection of organic compounds in aqueous media is of great interest since this would give the possibility, at least in principle, to detect low levels of organic pollutants in water or food

A simplified representation of the principle that underlies several molecular sensors derived from cyclodextrins is shown in Figure 5.1

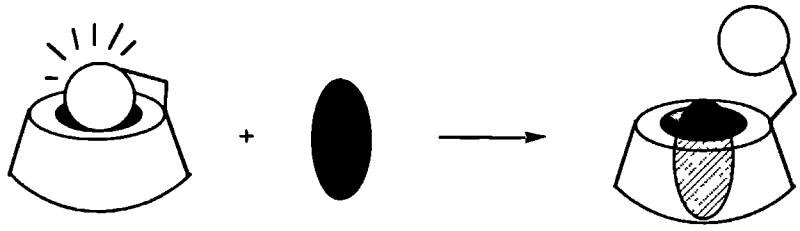


Figure 5.1. *Principle of a sensory system based on a cyclodextrin modified with a chromophoric group*

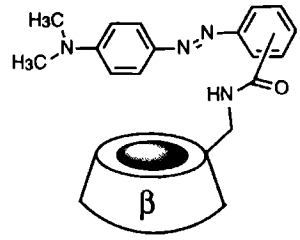
Such sensors consist of a cyclodextrin molecule that has been modified with an apolar chromophore. In aqueous solutions this apolar group is bound in the cavity of the cyclodextrin due to hydrophobic interactions. A guest molecule that is added to this solution can compete with the chromophoric group for binding in the CD-cavity. The latter is forced out of the cavity

into the polar aqueous solution which leads to a change in the solvation of the chromophore. This in turn gives rise to a change in the spectroscopic properties.

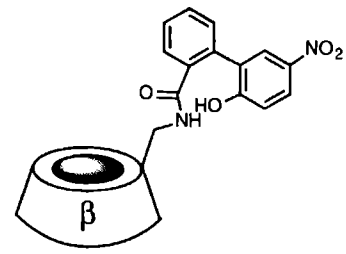
The sensory systems based on modified cyclodextrins can roughly be divided into three types which will be subsequently described in the next sections. These sensors differ in the spectroscopic method that is used for the detection of the guest binding. The first type is formed by cyclodextrins that are modified with UV/vis-active functions. These sensors show changes in their UV/vis-spectra upon guest binding. The second type is based on cyclodextrins that show variations in their circular dichroism spectra when a guest is bound. The third type of sensors makes use of changes in the fluorescence spectra of covalently bound chromophores to detect guest molecules. Several of these modified CDs have been studied with more than one spectroscopic method. For reasons of clarity, however, the literature examples in the following sections will be discussed on the basis of their most striking spectroscopic changes.

5.2 Sensors based on changes in UV/vis spectra

The pH indicator methyl red and a regioisomer of this compound have been covalently bound to β-CD to give the colour-change indicators **1a** and **1b**.



1 **a**: ortho
b : para



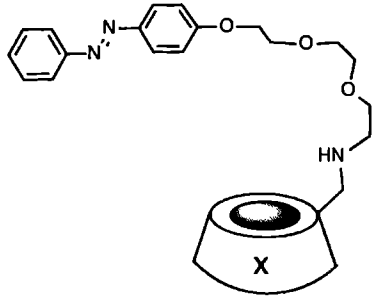
2

The latter compounds were synthesised starting from mono-(6-amino-6-deoxy)-β-cyclodextrin which was reacted with the corresponding acids in the presence of dicyclohexylcarbodiimide (DCC) as condensing reagent.[1, 2] These CD derivatives showed changes in their UV/vis spectra when guest molecules like *l*-borneol and adamantanecarboxylic acid (ACA) were added. In the case of compound **1a** the chromophore was bound in the CD cavity with an orientation parallel to the symmetry axis of the CD, whereas in the case of compound **1b** the methyl-red substituent was only partially encapsulated with an orientation perpendicular to the CD-axis. This difference in self-inclusion geometry can be observed by circular dichroism; it leads to a different response to guest molecules. The guest binding affinities of compound **1b** were

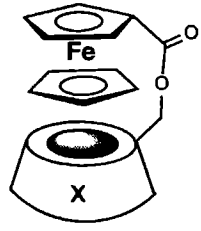
higher than those of compound **1a**, which is related to the fact that in **1a** the self-inclusion of the chromophore is less tight due to the para-substituent which is present in the methyl-red part. Compounds **1a,b** are not very soluble in water and therefore all measurements have to be performed in aqueous 10% ethylene glycol solutions in which concentrations of 1 x 10^{-5} M of ACA could be detected.[1] A disadvantage of these sensor compounds is that they require low pH-values and cannot be used in aqueous solution to detect organic molecules. Compound **2** was designed to overcome these problems. It was synthesised starting from mono-amino-functionalised CD, mentioned above, which was reacted with 6-nitro-3,4-benzocoumarin.[3] This sensor compound was more soluble in water and could be used to detect neutral organic molecules at pH 6.5 by following the changes in the UV/vis spectra upon addition of guest molecules like ACA or *l*-borneol. These changes are the result of the different pK_a-values of the bound and unbound form of the phenol part of the chromophore (ΔpK_a = 0.33 pH-units). Since for a complete disappearance of the colour at least a ΔpK_a of 3 pH-units is required, this cyclodextrin will not be a very promising sensor for the detection of guest molecules.

5.3 Sensors based on changes in circular dichroism spectra

A covalently linked achiral molecule containing a chromophore can induce a circular dichroism spectrum when it is bound in the chiral cavity of a cyclodextrin[4] If its location in the cavity changes upon the addition of a guest molecule this will be reflected in the circular dichroism spectrum, and the resulting spectral change can be used to calculate a binding constants.[5-7]



3 **a** : X = β
b : X = γ



4 **a** : X = β
b : X = γ

Following this principle Ueno et al. have attached azobenzene groups to β- and γ-CD to give compounds **3a** and **3b**, respectively.[8, 9] Compound **3a** showed changes in the circular dichroism spectra when neutral guest molecules like *l*-borneol and ACA were added. The

complex of **3a** with the latter guest, had a binding constant of 2160 M^{-1}. Upon photoirradiation, the azobenzene unit of **3a** underwent a trans-cis isomerisation reaction. In the photostationary state the amount of azobenzene moieties in the cis-form was approximately 85%. The binding affinity of the cis isomer for ACA appeared to be five times smaller than that of the trans-isomer for this guest molecule. This is probably due to the formation of a more stable self-inclusion complex when **3a** is in the cis-configuration than when it is in the trans-configuration.[9] Compound **3b** was tested as a sensor system for large organic molecules, e.g. cholic acid derivatives. The latter compounds could be detected with different sensitivities. Photoisomerisation of trans-**3b** by UV irradiation afforded the cis-configuration in 79%. As was observed for compound **3a** the host-guest binding constants of cis-**3b** were smaller (1.5 to 10 times) than those of trans-**3b**. Both **3a** and **3b** are nice examples of host molecules displaying a photo-induced control of guest binding. With these sensor molecules organic guests can be detected at concentration levels of approx. 1 x 10^{-4} M.
Other examples within this class of sensors are the ferrocene-appended cyclodextrins **4a,b**. These compounds also showed an induced circular dichroism spectrum due to the (partial) self-inclusion of the ferrocene unit in the CD-cavity. Upon addition of guest molecules the intensity of the circular dichroism bands decreased. The extent of this guest induced variation depended on the shape and size of the guest molecules and on the presence of functional (OH) groups. The detection limit was roughly proportional to the binding constant of the host-guest complex, although for steroidal compounds deviations were observed which were not fully understood.[6,7]

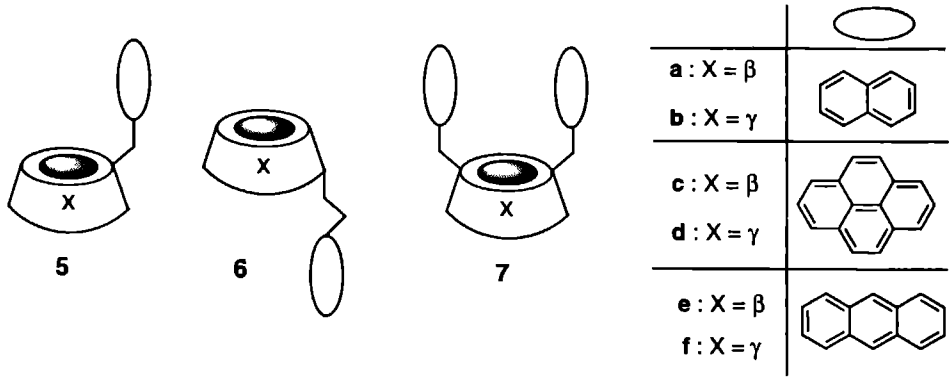
A general disadvantage of the host molecules described in the previous sections is that their ability to detect organic molecules is limited by the spectroscopic changes that can be measured by UV/vis or circular dichroism spectroscopy. Relatively high concentrations are required for these techniques when compared to fluorescence spectroscopy. The sensitivity of sensory systems can be improved by using fluorescent labels. In the next section several cyclodextrin derivatives which have been provided with such labels will be described.

5.4 Sensors based on guest induced changes in excimer emission

CDs with fluorescent labels were among the first CD-based sensor molecules that were developed. They have been intensively investigated by Ueno et al. [13-26] over the last 15 years. Cyclodextrins were modified with one or two naphthyl, anthracene or pyrene units. These CDs showed changes in their circular dichroism spectra upon the addition of guest molecules. This addition also changed their fluorescence spectra due to the formation or breakdown of excimers. Another fluorophore that has been covalently attached to a CD-unit is the dansyl

group. The fluorescence intensity of this group is highly dependent on polarity changes and therefore can be used to follow the guest binding process. The class of fluorophore-appended cyclodextrins can roughly be divided into two parts: one containing CDs with naphthyl, anthracene and pyrene groups and another one containing CDs with the dansyl group. In this section the first set of CDs will be described.

Pyrene is an excellent fluorescent probe because it easily forms excimers and displays a high fluorescent quantum yield. These excimers are the result of the formation of dimers between a pyrene molecule in the excited state and a pyrene molecule in the ground state. The resulting excited complex emits light at a higher wavelength than the excited monomer.[10] This excimer fluorescence is observed at high concentrations of pyrene but can also be induced by the addition of γ-CD to a diluted solution of this compound. This addition results in pyrene dimer formation inside the γ-CD cavity and leads to an increase of the excimer fluorescence and a decrease of the monomer fluorescence.[11] It was shown by Hamai that the dimer in fact is a 2:2-complex which results from the association of two 1:1 (pyrene:CD) complexes.[12] The same dimer and excimer formation is possible with naphthalene and anthracene derivatives instead of pyrene. Several sensors (**5-7**), which are schematically presented below, were synthesised and examined with respect to their ability to detect guest molecules by changes in the excimer emission. The specific place of substitution of the chromophores and the exact structure of the spacers can be found in references 13-26.



Compounds **5-7** can form a great variety of inter- and intra-molecular complexes which are schematically presented in Figure 5.2. Excimer emission was observed when naphthalene was added to an aqueous solution of compound **5b** indicating the formation of a 1:1 complex of type I. Addition of *l*-borneol to this solution caused a decrease of the excimer fluorescence and

an increase of the monomer fluorescence intensity. This is due to the displacement of the naphthalene molecule by *l*-borneol.[13] Molecule **5b** could also be used to detect several ketones. These guest molecules, also formed a complex of type I. In this complex an efficient quenching of the fluorescence of naphthalene by the ketone was observed, which could be suppressed by the addition of *l*-borneol, indicating that the quenching process is the result of the complex formation.[14]

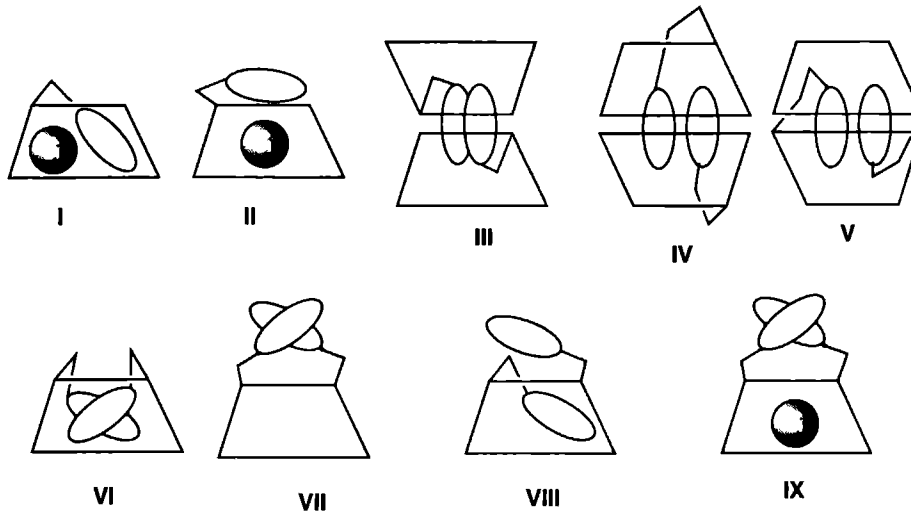


Figure 5.2 *Schematic drawings of inter and intra molecular complexes between guest molecules and cyclodextrins containing fluorescent probes.*

To get more information about the requirements for a sensor system in which pyrene is used as the fluorophore, several derivatives of molecule **5d** and **6d** were prepared which varied in the position of the pyrene unit and the type of linker between this probe and the CD moiety. For example compound **6d** in which the pyrene unit is directly linked to the secondary side of the CD via an amide bond (at C-3 of an inverted glucose (altrose) unit) showed higher affinities towards large guest molecules than the corresponding compound **5d**.[15, 17] This indicates that binding of large substrates preferentially takes place via the secondary side. Compound **6d** displayed a tendency to dimerise to give a complex of type V which leads to excimer fluorescence.[15] This excimer emission was influenced significantly by the position of the pyrene moiety relative to the CD-cavity. For example, γ-CD which was esterified at the C-2 position with pyrenecarboxylic acid, mainly showed excimer fluorescence, whereas the analogous C-3 derivative displayed the fluorescence of the monomer.[20] The direct linking of a pyrene unit to the primary side did not lead to excimer formation,[15] although a type III complex might have been expected. If however a spacer was introduced between the CD unit of compound **5d** and the pyrene moiety an excimer emission was observed due to the formation of

a complex of type IV. The formation of this complex was found to be pH dependent, which can be explained as follows. At higher pH values (>12.7) the hydroxyl groups at the C-2 position of the CDs become deprotonated which will lead to an electrostatic repulsion between the two γ-CD moieties. As a result the dimeric complex will dissociate which causes a decrease of the excimer fluorescence.[19] The dimeric complex also dissociated if guest molecules (like steroids) were added to the solution. The decrease in excimer emission and the increase in monomer emission were used to detect a large variety of steroids in the concentration range of 10^{-4} M.[18] The β-CD analogue **5c**, in which the pyrene group was also linked via a long spacer to the CD ring showed no excimer fluorescence, probably because its cavity was too small to encapsulate two pyrene moieties.[16]

Anthracene has also been used to modify CDs, e.g. see compounds **5e** and **5f**.[21] In solution compound **5f** dimerised to give a complex of type III as was concluded from UV/vis studies. Although this complex could have given excimer emission, fluorescence studies only revealed the presence of monomer emission bands. This result was explained by assuming either an interaction between the excited anthracene moiety and the carbonyl group of the ester linkage or the formation of a photodimer from the excimer. In both cases an excimer emission will be absent.[21] Upon addition of guest molecules to a solution of compound **5e** or **5f**, an increase in the fluorescence intensity was observed due to interactions between the guest and the fluorescent probe. In the complex, the latter probe was believed to be positioned as a cap over the CD (complex of type II in the case of **5e** and of type I in the case of **5f**). A very elegant synthesis of a CD dimer has been achieved by performing a photo dimerisation reaction on compound **5f**.[21] The resulting γ-CD dimer was not used in binding studies but might possess interesting co-operative binding properties.

Another approach to achieve guest induced changes in the excimer fluorescence is the covalent attachment of two chromophores to one CD moiety. The synthesis of compounds of this type starts from capped CDs.[27] Several regioisomers of compound **7b** could be prepared in which the flexibility of the linker between the naphthyl unit and the CD proved to be important for achieving excimer emission. If this unit was linked via a thioether bond, no excimer emission was observed because the two naphthyl chromophores were not able to interact with each other in a face-to-face orientation.[22] Compounds **7b**, however, showed an increase in fluorescence intensity upon guest binding, which suggests that the naphthyl units are acting as caps on the primary side of the CD (type II complex). If the naphthyl group of compound **7b** was connected via an ester bond to γ-CD, the main fluorescence signal was the excimer emission. Upon addition of guest molecules this excimer fluorescence did not change. This result was explained by assuming that initially a complex of type VI was present which upon binding of a guest molecule was transformed into a type IX complex. Both complexes must have had rather

similar quantum yields. The occurrence of the latter type of complex was proven by circular dichroism.[25]

An improvement in the sensitivity of the sensor systems could be made by using β-CD instead of γ-CD as the host compound. Compound **7a** showed a mixture of monomer and excimer emission which is due to the presence of both type VII and type VIII complexes. The formation of a type VI complex was not possible due to the small size of the β-CD cavity. Upon addition of guest molecules the excimer fluorescence intensity increased while the monomer intensity decreased indicating that the mixture of type VII and VIII complexes was transfered into a type IX complex. The guest induced variation in the monomer and excimer fluorescence of **7a** was used to detect several organic compounds at concentration levels of 10^{-5} M.[24,26] The changes in the excimer fluorescence were also used to calculate binding constants.[23]

Another bis-functionalised γ-CD is compound **7d**, which contains pyrene as the fluorophore. All four regioisomers of this cyclodextrin showed monomer and excimer fluorescence. The guest induced changes in the excimer emission were used to determine the binding constants for several guest molecules, which were found to be very different for the CD regioisomers. For example, the AC-regioisomer showed hardly any response towards *l*-borneol, whereas the AD and AE-regioisomers responded dramatically. Addition of *d*-menthol to a solution of the AD-isomer led to an increase of the excimer fluorescence whereas the addition of deoxycholic acid led to a decrease. This remarkable phenomenon was explained by assuming different binding geometries for each of the host-guest complexes.[28]

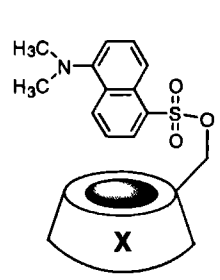
Compound **7f** is a bis-functionalised CD with anthracene as the fluorophore. Circular dichroism spectra revealed that both anthracene units are included in the γ-CD cavity. They are expelled from this cavity upon the addition of adamantane carboxylic acid to give a type IX complex. The self-inclusion of the anthracene units however was so strong that compound **7f** could not be used as a sensor.[21]

Most of the compounds described in this section have the disadvantage that the fluorescence changes in the monomer emission upon guest binding are small. Also the changes in the excimer emission are difficult to predict and are usually too small to determine a binding constant. To overcome this problem dansyl-modified CDs have been synthesised.

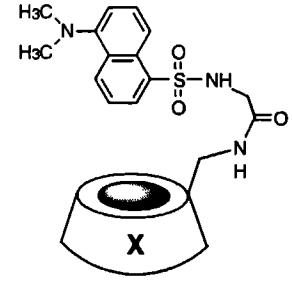
5.5 Dansyl-modified cyclodextrins

Fluorophores whose emissions are sensitive to the local environment are of great interest for the development of new sensors. The dansyl (5-dimethylamino-1-naphthalenesulfonyl) group is such a fluorophore since its fluorescence emission intensity changes in respons to the polarity of the environment. In a polar solvent, such as water, the fluorescence emission is relatively

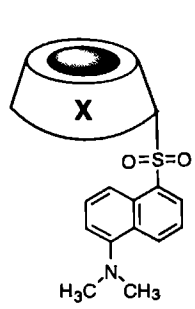
weak, whereas in nonpolar solvents the emission intensity is higher and the emission wavelength is blue shifted. This increase in fluorescence intensity also occurs when the dansyl group is bound in the apolar cavity of a β-CD and this phenomenon was used to determine the binding constant of the complex with dansylamine.[29] The dansyl group can be excited at a wavelength longer than 350 nm, where many organic compounds have no absorption, its Stoke shift is large and its fluorescence is not susceptible to oxygen quenching. These properties make dansyl derivatives attractive to work with. With this in mind Ueno and coworkers prepared the following dansyl appended CDs (**8**-**10**).



8 **a** : X = β
b : X = γ



9 **a** : X = β
b : X = γ



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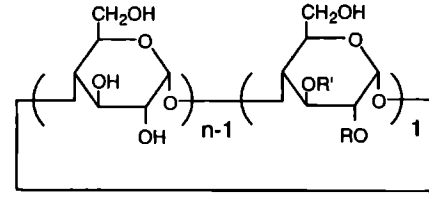


10

	X	R	R'
a	β	**dansyl**	**H**
b	β	**H**	**dansyl**
c	γ	**dansyl**	**H**
d	γ	**H**	**dansyl**

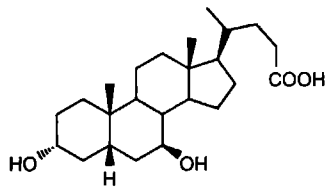
Reaction of monoamino-functionalised β- and γ-CDs with dansylglycine gave compounds **9a** and **9b**.[30,31] The other dansyl derivatives were all obtained by a direct reaction of dansyl chloride with the appropriate cyclodextrin. The reaction conditions were chosen in such a way that only the desired compound was formed. Compounds **8** were found to be more accessible

than compounds **10**, which decomposed very easily during the reaction yielding the corresponding epoxides. Both the 2- and 3-functionalised regioisomers were formed which could be separated by reversed phase HPLC.[32, 33] Al these appended CDs were tested for their ability to detect guest molecules. A sensitivity factor (Sf) was introduced which describes how suitable the sensor molecule is for detecting a certain guest. This Sf is defined as:

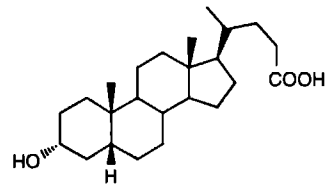
$$Sf = \frac{\Delta I}{I_0}$$

in which ΔI is the change in the intensity of the observed fluorescence signal at a certain host-guest ratio (e.g. 1:10) and I_0 is the intensity of the signal before the addition of the guest molecule. If Sf-values, determined at the same ratio, are compared it is important that also the concentrations of the host molecules are the same, since at higher concentrations of the latter molecules the ratio between bound and unbound guest molecule will increase. Furthermore, care has to be taken when comparing Sf-values because they depend on whether the fluorescence signal increases or decreases. In systems where the observed signal decreases upon the addition of guest molecules the maximum Sf-value will be 1 whereas in systems in which this signal increases the Sf-values can be higher than 1, which does not necessarily mean that the sensor molecule is more sensitive.

Compounds **9a** and **9b** showed a decrease in fluorescence intensity upon the addition of guest molecules. A 10% DMSO solution in water was used to avoid solubility problems. Sf-values were determined at a host concentration of 2.25 x 10^{-6} M and at a host:guest ratio of 1:45 for steroids and 1:450 for smaller guest molecules like *l*-borneol. The guest molecule ursodeoxycholic acid was detected with Sf-values of 0.63 and 0.21 using compounds **9a** and **9b**, respectively. This observation is quite strange since it was expected that the large steroid molecule would fit better in the γ-CD than in the β-CD ring as was observed for lithocholic acid which only differs from ursodeoxycholic acid by the presence of one hydroxyl group.[31]



Ursodeoxycholic acid



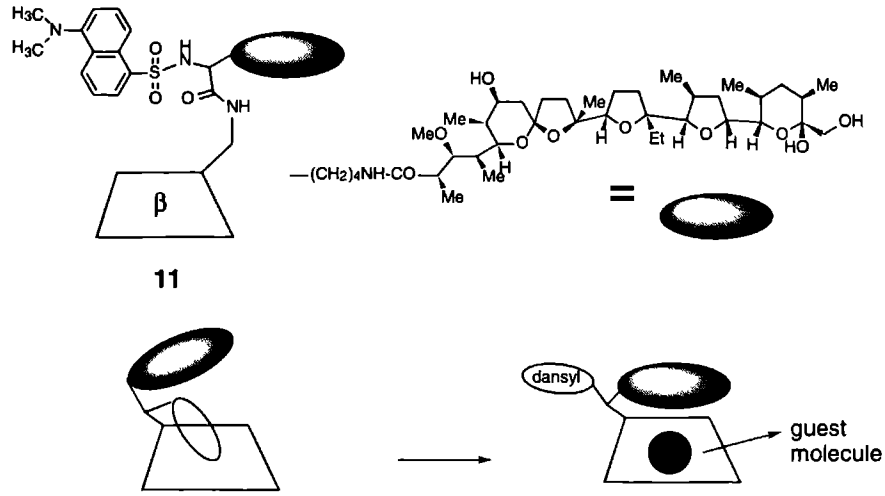
Lithocholic acid

Lithocholic acid could be detected very efficiently using compound **9b** at a host:guest ratio of 1:4, the Sf-value being 0.194.[31] Compound **9a** was able to detect several small molecules (*l*-borneol, *l*-fenchon) but larger concentrations of guest molecules (host:guest = 1:450) were required to reach sufficiently high Sf-values (up to 0.45). Compound **9b** appeared to be less suitable for this purpose as could be concluded from the Sf-values which were smaller than 0.06. Remarkably, this host molecule showed an *increase* in fluorescence intensity upon the addition of cyclohexanol and *l*-menthol leading to negative Sf-values down to - 0.02. This guest-induced increase of the fluorescence intensity suggests that the dansylglycine unit is included in the γ-CD cavity together with the flat guest molecule forming a complex of type I (Figure 5.2).

The three regioisomeric compounds **8a**, **10a**, and **10b** are more soluble in water than the other dansyl containing CDs and therefore binding studies could be performed in pure buffer instead of in a 10% DMSO-solution.[33] Sf-values were determined at a host concentration of 1 x 10^{-5} M and at a host:guest ratio of 1:10, which makes a comparison with the above described compound **9a** difficult. All compounds showed a decrease in fluorescence intensity upon guest binding, indicating that the dansyl group is removed from the cavity by the guest molecule. The latter was also reflected in a red shift of the maximum emission wavelength that occured upon guest binding. Many guest molecules could be detected with compounds **10a** and **8a** and with very good Sf-values (up to 0.85). A clear relationship was found between the binding constants and the Sf-values of the guest molecules. Compound **10b** could not be used as a sensor for organic molecules since the dansyl moiety was included too strongly in the CD unit.
Measurements with the three regioisomers of γ-CD, compounds **8b**, **10c** and **10d** [32] were performed at host concentrations of 5 x 10^{-6} M and at a host:guest ratio of 1:20 and 1:200 for steroidal compounds and for small guest molecules (like *l*-borneol), respectively. For compound **10c** Sf-values of 0.62 and 0.25 were found for ursodeoxycholic acid and *l*-borneol, respectively. These values are similar to the values measured for the β-CD analogues but since a higher host:guest ratio was used we can conclude that the γ-CD derivatives are less suitable sensor molecules than the corresponding β-CD derivatives. A remarkable feature of the three above mentioned regioisomers of γ-CD is their different sensitivity towards guest molecules, which was not observed in the β-CD series. This phenomenon may be related to the larger and more flexible cavity of the γ-CD analogues, which can lead to more diverse interactions with the guest molecules.

Very recently β-CD derivative **11** was described which contains a dansyl group to which a monensin moiety is attached.[34] This triad system can be used for the detection of alcohols. Monensin is an ionophore. Its structure can be changed from an open form to a closed macrocyclic form when it binds a sodium ion. The monensin-sodium complex forms a

hydrophobic cap on the CD which enhances the binding affinity for various alcohols up to a factor of seven. The binding geometry is depicted below.



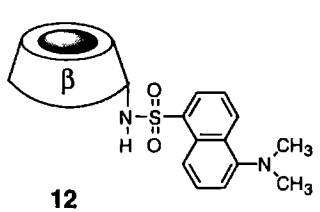
11

All sensor systems described above, were tested in homogeneous aqueous solution, which makes their regeneration troublesome. To overcome this problem Prasad et al. [35] have entrapped compound **9a** in an inorganic porous sol-gel matrix. This matrix was brought in contact with an aqueous solution of borneol (1 mM) and the fluorescence intensities of the incorporated molecule **9a** were measured before and after exposure to this alcohol. The binding of borneol resulted in a 40% decrease of the fluorescence intensity. Since a reference sol-gel, containing only dansylglycine, showed a very small response to borneol, it was concluded that this molecule expels the dansyl group from the CD cavity leading to a decrease of the fluorescence intensity. The regeneration of the system was achieved by immersing the film in ethanol to remove the borneol. To our knowledge, this is the only example of an immobilised fluorophore appended CD reported to date. The development of sensor systems that can be regenerated is very important for future applications. Another important requirement is that the sensor device is chemically stable under slightly acidic or basic conditions. CDs that are functionalised with dansyl moieties at their secondary side, e.g. **10a-d**, give higher Sf-values than those functionalised at their primary side as was discussed above.[33] All secondary side functionalised CDs reported in the literature, however, lack the stability required for a good sensing device. For instance CDs **10a-d** are easily decomposed into the corresponding epoxides upon treatment with base. This prompted us to develop more stable CD derivatives in which the dansyl group is linked to the secondary side by means of a sulfonamide function. The synthesis and sensor properties of these compounds will be described in the following sections.

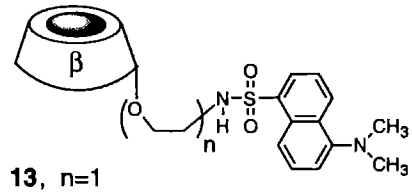
5.6 Results and discussion

5.6.1 Synthesis of dansyl modified cyclodextrins

We designed the dansyl functionalised cyclodextrins **12-14** which differ in the length of the linking spacer. If the dansyl group is displaced from the CD cavity by a guest molecule this will

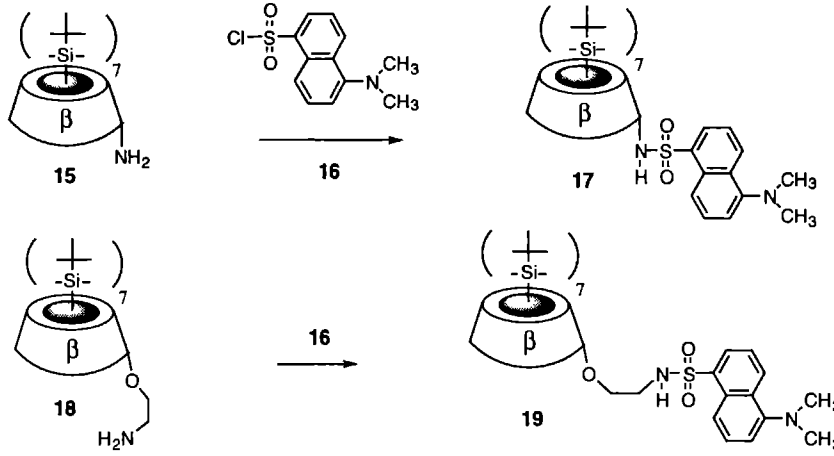


12



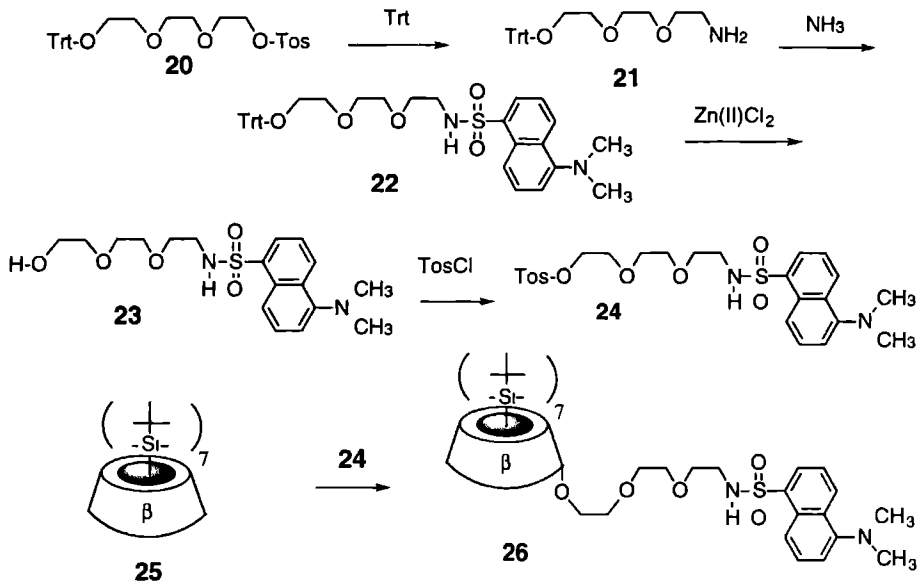
13, n=1
14, n=3

lead to a change in the local environment of this group, and in turn to a change in the intensity of the fluorescence spectrum. We expected that by lengthening of the linking chain the fluorescence intensity change would become larger since the chromophore is more free to move away from the cavity. For compound **14** we chose the more polar oligoethyleneglycol spacer rather than an alkyl spacer since the latter can be included in the CD cavity itself (as was observed for our CD dimers, see Chapter 2). This self-inclusion will lead to a lower sensitivity for guest molecules and, therefore, should be avoided.



Scheme 5.1

The synthetic routes to compounds **12-14** are given in Schemes 5 1-5 3 The preparation of the intermediates **17** and **19** was very straightforward and started from compounds **15** and **18**, respectively (see Chapters 2 and 3 for their syntheses) The latter compounds were reacted with 0 95 equivalents of dansyl chloride **16** to give the silylated target molecules in reasonable yields (58 and 59%, respectively) after column chromatography (Scheme 5 1) More reaction steps were required to obtain the silylated compound **26** (Scheme 5 2)

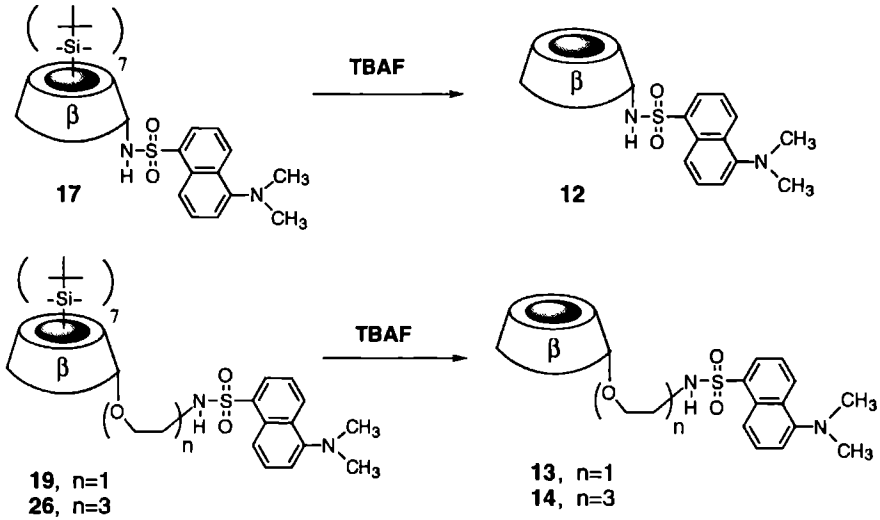


Scheme 5.2

Compound **20**, which was described in Chapter 4, was treated with ammonia gas in ethanol using an autoclave, to yield compound **21** in 72% yield The latter amine was coupled to dansyl chloride and the resulting compound **22** (48% yield) was deprotected with $ZnCl_2$ in dichloromethane/methanol to give compound **23** in 72 % yield After tosylation (66% yield) with tosyl chloride in pyridine compound **24** was isolated as a yellow oil The yields of the reactions leading to compound **24** were slightly lower than expected for these type of conversions This is probably due to the presence of a trace of water which could not be removed easily No effort was made to improve the yields since enough product was obtained for the synthesis of **26**

The silylated CD **25** was deprotonated with sodium hydride in refluxing THF and the resulting alkoxide reacted with 0 6 equivalents of compound **24** to give compound **26** in 33% yield (calculated from **24**) after purification by column chromatography

Desilylation of the protected compounds **17**, **19** and **26** was achieved with tetrabutyl-ammonium fluoride (TBAF) in refluxing THF. The compounds were purified by dissolving them in a small volume of ethanol/water (2/1, v/v) followed by precipitation with ethyl acetate. Further purification (removal of the last traces of the TBA-salts) was achieved using either cation-exchange chromatography or exclusion chromatography. The desilylation reactions gave compounds **12-14** in good yields (63, 76 and 79%, respectively). All three compounds were fully characterised by elemental analysis and spectroscopic methods.



Scheme 5.3

5.6.2 Fluorescence studies at pH 7

Compounds **12-14** were studied with respect to their ability to detect the guest compounds adamantanecarboxylic acid (ACA) and cyclohexanol (CH). The emission spectra of the host compounds were recorded at various concentrations of ACA and CH. Addition of the guest resulted in a decrease of the fluorescence intensity. This guest induced variation of the emission intensity was used to measure the binding constants. Also the Sf-values at a host:guest ratio of 1:10 were determined ([host]= 1 x 10^{-6} M). The results are summarised in Table 5.1. For comparison the values reported in the literature [33] for compound **10a** are also presented. This table reveals that the binding constants of the complexes with **12-14** are smaller than those of the literature compound **10a**. For compound **12** the changes in emission intensity were too small to calculate accurate values of K_b and Sf. This indicates that the environment of the dansyl group in this host molecule does not change significantly. This may be caused by a very

strong self-inclusion effect or it may be the result of the fact that the dansyl group is not included in the CD cavity at all.

Table 5.1 *Binding constants and sensitivity factors for complexes between dansyl functionalised cyclodextrins and guest molecules* [a]

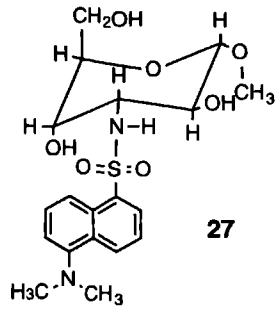
Host molecule	Guest molecule			
	cyclohexanol		adamantane carboxylic acid	
	K_b (M^{-1})	Sf	K_b (M^{-1})	Sf
12	-	-	-	-
13	20	0.0001	10400	0.003
14	70	0.0006	6300	0.022
10a [b]	582	0.041	28000	0.65

[a] 0.05 M phosphate, 0.05 M citric acid, buffered at pH 7.0, [host] = 1 x 10^{-6} M, Sf-value determined at host:guest = 1:10. [b] Sf-value determined at [host] = 1 x 10^{-5} M and host:guest = 1:10 (see ref.33).

The Sf-values of **13** and **14** were smaller than we expected on the basis of the values reported for compound **10a**. Since this also might be the result of a strong inclusion of the dansyl unit in the CD cavity, we investigated this self-inclusion process in more detail.

5.6.3 Self inclusion of dansyl-modified cyclodextrins.

Comparison of the maximum emission wavelength (λ_{max}-value) of compounds **12-14** can give information about the polarity of the environment of the dansyl group. The λ_{max}-values observed for compounds **13** and **14** (519 nm and 520 nm, respectively) are lower than the value observed for compound **12** (526 nm). This may indicate that the dansyl groups of the former two compounds are more shielded from the aqueous solution, due to a deeper inclusion inside the CD cavity, than the dansyl group of compound **12**. The values of compounds **13** and **14** are within error identical to the value (520 nm) reported for compound **10b** which has a dansyl group that is deeply and completely buried inside the β-CD cavity.[33] This comparison, however, is not completely correct since in the latter compound the dansyl moiety is connected via an ester bond instead of an amide bond. We therefore synthesised the reference compound **27** which showed a λ_{max}-value of 530 nm which may be taken as a standard for a non-bonded, amide linked dansyl moiety.



27

Since compound **12** shows an emission band at more or less the same wavelength as compound **27** we may tentatively conclude that its dansyl moiety is not deeply buried in the CD-cavity or even is located outside this cavity. To get more information about the self-inclusion of the dansyl groups in our compounds, we determined the pK_a-values of their dimethylamine functions. We expected that the inclusion of the dimethylamino function would result in a decrease of the pK_a-value. It is known that the UV/vis spectra of dansyl derivatives are different for the free base form and the protonated form,[36] which can be used to determine the pK_a-values. For the neutral forms of compounds **12-14** absorption maxima were observed at 248 nm and 333 nm. The intensity of the absorption bands at these wavelengths decreased upon lowering the pH while at the same time the intensity of a band of the protonated form at 286 nm increased. Isosbestic points were observed at 231, 268 and 306 nm indicating that the protonated and the deprotonated forms interconverted directly. From the changes in absorption at 286 nm and 333 nm as a function of pH, titration curves (e.g. Figure 5.3) were constructed which were used to calculate the pK_a-values. The results are summarised in Table 5.2.

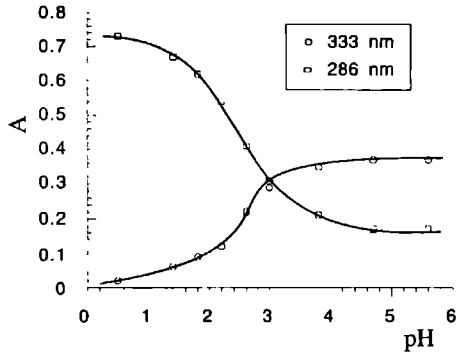


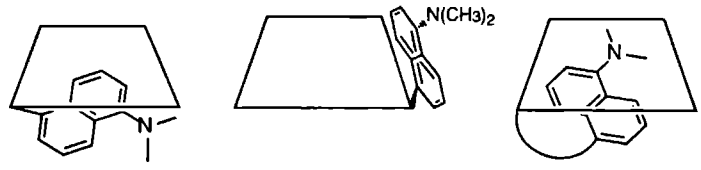
Figure 5.3 *pH-Titration curve of host molecule **14** (9.7×10^{-5} M). Shown are the changes in absorption at 286 nm (H^+-form of **14**, squares) and at 333 nm (neutral form of **14**, circles).*

Table 5.2 *pK_a-values of the dimethylamino functions of dansyl appended cyclodextrins* [a]

Cyclodextrin	pK_a-value (± 0.1)	λ_{max} (nm) [b]
12	4.0	526
13	1.5	519
14	2.5	520
dansylamide [c]	3.9	580

[a] Determined by UV/vis spectroscopy, [CD] = 9.7 x 10^{-5} M, 0.05 M Phosphate, 0.05 M citric acid, buffered at pH 7.0. [b] Maximum emission wavelength of the cyclodextrin derivative. [c] Taken from reference 36.

The measured values reflect the accessibility of the dimethylamino group and, therefore, can be used as an indication of the shielding of this group by the CD cavity. Compound **12** shows a pK_a-value which is similar to the reported value of free dansylamide. This confirms the conclusion from the fluorescence studies, *viz.* that the dansyl moiety of **12** is either partly included in the CD-cavity (structure X) or located outside the cavity (structure XI). The pK_a-values of compounds **13** and **14** are significantly smaller than the pK_a-values of **12**. This result, in combination with the result from the fluorescence studies, suggests that the dansyl units of compounds **13** and **14** are completely encapsulated by the CD-rings (complex of type XII).

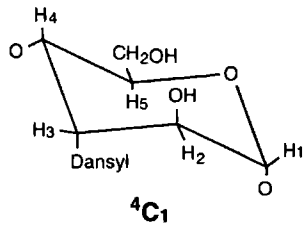


X XI XII

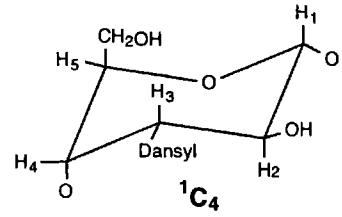
To further investigate this self-complexation behaviour we also determined the fluorescence lifetimes of compounds **12** and **13** at pH 7 using the picosecond single photon count (SPC) technique.[37] The lifetime (τ) for compound **13** could be obtained by a monoexponential fitting of the fluorescence decay curve which gave a value of 8.3 ns. For compound **12** the fluorescence decay could be analysed by assuming two lifetime components (τ_1 and τ_2) which attributed to the decay with relative contributions C_1 and C_2. The calculated values were τ_1 2.3 ns (C_1 = 0.66) and τ_2 4.6 ns (C_2 = 0.34). These lifetimes suggest that the fluorophore in compound **12** is in a more polar environment than in compound **13** which would be in

agreement with the type of complexes described above In the following we will discuss the structure of molecule **12** in more detail

The dansyl group of **12** is attached at the C-3 position, which means that two conformations of the modified glucose (altrose) unit are possible $^4C_1$ and $^1C_4$



$^4C_1$



$^1C_4$

CPK models suggest that in the case of a $^4C_1$-conformation the dansyl group can be encapsulated by the CD-unit, yielding a type X complex, but not in the case of a $^1C_4$-conformation In the former conformation the dansyl group is in an axial position, which is energetically unfavourable The energy required to attain this conformation, however, can be provided by the self-inclusion process (as was seen for the CD dimers in Chapter 2) A study of the ^{1}H-NMR-spectra of compound **12** in D_2O was undertaken to get information about the conformation of the altrose unit Since the H-1^A signal at δ = 4 70 ppm overlapped with the HDO signal, the temperature was gradually increased from roomtemperature to 50 °C (which resulted in a shift of the HDO signal) allowing the determination of the J_{12}-coupling constant, which amounted to6 1 Hz This value is in accordance with a diaxial coupling indicating a $^1C_4$-conformation which makes the existence of a type X complex unlikely An interesting observation in the ^{1}H-NMR spectrum of compound **12** was the broadening of one of the signals of the H-1 protons This signal was shifted upfield (δ = 4 4 ppm) Upon heating this signal moved to lower field This suggests that the dansyl group is located *outside* the CD-cavity (type XI complex) in close proximity to one of the H-1 protons, which are at the outside of the CD-ring The fact that the H-1 signal at 4 4 ppm shifts to lower field upon heating, is in agreement with such a conformation, since at higher temperatures the shielding by the dansyl moiety will be less effective because this moiety can turn away from the H-1 site more easily
The two lifetime components which were necessary to describe the fluorescence decay of compound **12**, indicate that the dansyl group has two different binding geometries in the $^1C_4$-conformation In other words there must be two kinds of type XI complexes which is not unlikely according to CPK models

5.6.4 Fluorescence studies at pH 1

As shown above (Table 5.1) sensor molecules **13** and **14** have very low Sf-values probably as a result of the tight binding of the dansyl moiety inside the CD cavity. To overcome this problem, we decided to lower the pH which leads to the protonation of the dansyl group, resulting in a less tight self-inclusion of this group, and hence to a better response towards guest molecules.

Since it is known that pyrene appended CDs can dimerise in solution (see Section 5.4) we first investigated whether our host molecules formed dimers or aggregates in water at higher concentrations. The emission intensity of the CD-molecules was monitored as a function of their concentration (1 x 10^{-7} to 1 x 10^{-5} M) at pH 1 and at pH 7.0 (1 x 10^{-7} to 2 x 10^{-6} M). All three compounds showed a linear correlation indicating that no aggregation occurred under these conditions. At concentrations above 3 x 10^{-5} M at pH 1.0 deviations from linearity were observed for compounds **12** and **14** (cuvette 0.500 x 1.000 cm) due to inner filter effects.[38] For compound **13** this deviation was already observed above concentrations of 1 x 10^{-5} M at pH 1.0, probably due to the higher quantum yield of the fluorescence of this compound.

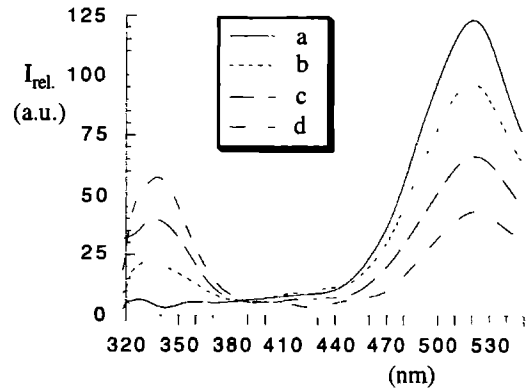
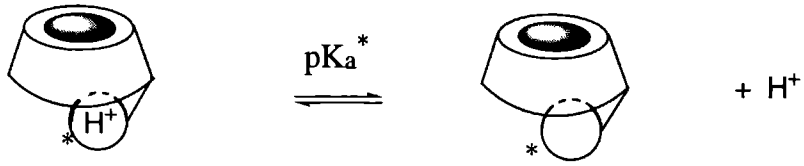


Figure 5.4 *Corrected fluorescence spectra of compound **14** (1 x 10^{-5} M, λ_{exc} 306 nm) in water of different pH's; (a) pH 3.89; (b) pH 2.51; (c) 1.92; (d) 0.58.*

In Figure 5.4 the fluorescence spectra of compound **14** at different pH-values are presented. It can be seen that two emission bands are present at low pH which can be ascribed to the protonated (335 nm) and the deprotonated (520 nm) form of this compound.[36] According to the UV/vis spectrum all molecules were protonated at pH 0.6 (not shown). The fluorescence

emission at 520 nm therefore must be the result of the following excited state equilibrium:



The pK_a-values in the singlet excited state (pK_a^*) of dansyl derivatives can be very different from the normal pK_a-values. For example, the pK_a^*-value of dansylamide has been calculated to be -14.[36] This means that excitation of compound **14** at pH 0.6, will lead to deprotonation of the fluorophore which results in an emission signal of the neutral form at 520 nm.

The fluorescence lifetimes for the dansyl signals at 335 nm of compound **13** and for reference also for compound **12** were determined at pH 1.0. Proton transfer in the excited state could not be observed with the picosecond SPC technique probably because this process is too fast.[39]
For compound **12** again two lifetime components were required to describe the fluorescence decay at 335 nm. The calculated values amounted to τ_1 0.26 ns ($C_1 = 0.55$) and τ_2 4.8 ns ($C_2 = 0.45$). The shorter lifetime component may result from protonated dansyl groups that are located in the aqueous solution far from the CD moieties, while the longer component probably arises from the protonated dansyl group of molecules in the type XI conformation.
For compound **13** at pH 1.0 the following lifetimes were observed (fluorescence band at 335 nm): τ_1 0.3 ns ($C_1 = 0.13$) and τ_2 7.9 ns ($C_2 = 0.87$). The shorter lifetime component is due to a similar process as discussed for compound **12** but the relative contribution of this process is smaller than for compound **12**. This is in agreement with the observed pK_a-values and the proposed conformations of **12** and **13**. The longer lifetime component observed in the case of compound **13** is probably caused by protonated dansyl units that are located inside the cavities of type XII complexes.

The fluorescence lifetimes belonging to the signals at 520 nm are difficult to interpret. One of the reasons may be that they originate from more than two processes, some of which are drawn in Figure 5.5.

When the fluorescence decay of **13** was monitored at 520 nm (pH 1.0), lifetimes (τ_1 0.8 ns ($C_1 = 0.06$) and τ_2 7.9 ns ($C_2 = 0.94$)) comparable to those determined at 335 nm were observed. The fact that for both emission bands (at 335 and 520 nm) the same τ_2 values were measured may indicate that the protonated and the deprotonated forms of **13** are in fast equilibrium. The

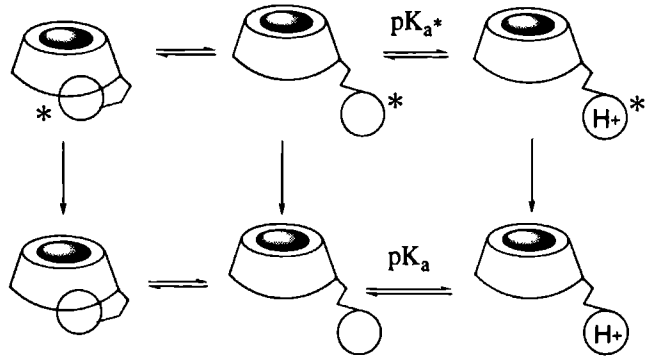


Figure 5.5 *Possible processes of fluorescence decay in the case of compound 13*

fluorescence decay of compound **12** monitored at 520 nm, could be described with τ_1 2.4 ns and τ_2 10.7 ns. The relative contributions were dependent on the precise wavelength of detection (500-540 nm) and varied between 20 and 50% for C_1 and between 80-50% for C_2 indicating the presence of two emission bands between 500-540 nm.

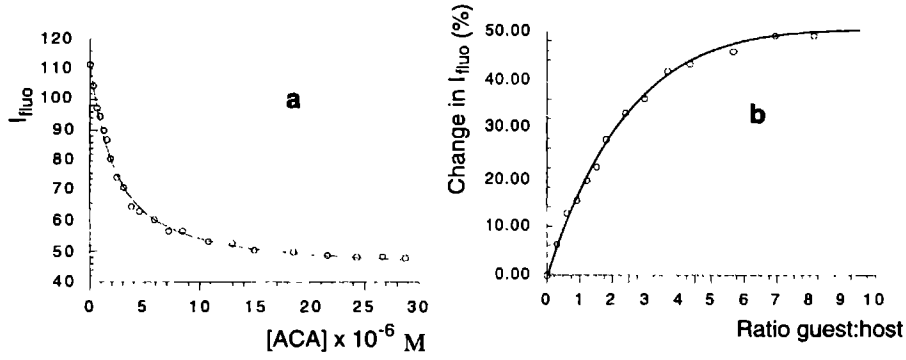


Figure 5.6 *(a) Fluorescence intensity at 520 nm of compound 13 as a function of the concentration of ACA and (b) Change in fluorescence signal as a function of the ratio ACA:13, pH 1.0, [13] = 1.0 x 10^{-6} M.*

Binding studies were performed with compounds **12-14** and the guests cyclohexanol and ACA at pH = 1.0. The fluorescence band at 335 nm increased upon guest binding, whereas the band at 520 nm decreased. Apparently, binding favoured the formation of the protonated form of the CD-derivative, from which we may conclude that the deprotonated form is stabilised by self-complexation. Since the intensity decrease at 520 nm was larger than the intensity increase at 340 nm, the band at the former wavelength was used to calculate binding constants. A typical

titration curve is given in Figure 5 6 a The determination of Sf-values was performed using a procedure in which the host guest ratio was plotted as a function of the fluorescence change (see Figure 5 6 b) The values for Sf and K_b are summarised in Table 5 3 As can be concluded from this table, compound **12** does not show any changes in fluorescence signals at pH = 1 0 This result is in agreement with what was found above, viz that the dansyl group is located outside the cavity of the CD ring

Table 5.3 *Binding constants and sensitivity factors determined for complexes between cyclodextrins **12-14** and guest molecules at pH 1 0* [a]

Host molecule	Guest molecule: cyclohexanol K_b (M^{-1})	cyclohexanol Sf (10^{-5} M)[b]	adamantane carboxylic acid K_b (M^{-1})	adamantane carboxylic acid Sf (10^{-5} M)[c]	adamantane carboxylic acid Sf (10^{-6} M)[c]
12	-[d]	-[d]	-	-	-
13	540 ± 30	0 017	(4 5 ± 0 5) x 10^5	0 61[e]	0 52
14	450 ± 30	0 020	(2 2 ± 0 2) x 10^5	0 57[e]	0 38
10a[f]	582	0 041	2 8 x 10^4	0 65	-

[a] Sf-values were determined at a host guest ratio of 1 10 using host concentrations of 10^{-5} or 10^{-6} M [b] Estimated error is 10 % [c] Estimated error is < 2 % [d] No changes in fluorescence intensities were observed for this compound [e] Determined at a host guest-ratio of 1 3 Smaller ratios did not result in significantly larger changes in fluorescence [f] Taken from reference 33, data were obtained at pH 7

Both CH and ACA are detected by compounds **13** and **14** with much higher Sf-values at pH = 1 0 than at pH 7 (compare Tables 5 3 and 5 1) The Sf-values at low pH are in the same range as the values reported in the literature for reference compound **10a** This comparison is not totally valid for the substrate ACA since our experiments were performed at lower pH than those in the literature and ACA is known to bind much more strongly at lower pH than at higher pH [40] Our Sf-values, however were determined at a lower host guest ratio (1 3), which allows us to conclude that we have realised a more sensitive system Figure 5 6 b reveals that 0 5 equivalent of ACA (which corresponds to a concentration of 5 x 10^{-7} M) results in a 10% fluorescence change Our system is the first fluorophore appended CD that is able to detect adamantanecarboxylic acid at such a low concentration

The idea of using a longer spacer, as in compound **14**, was to increase the changes in the fluorescence intensity of the host upon binding of a guest Since the Sf-values of **14** for both guests are the same within experimental error as those of **13**, we can not conclude that a longer spacer has a positive effect This might be due to the fact that the ethyleneglycol chains can be encapsulated in the cavity of **14** [41] and thereby blocking the cavity for complexation of the guest molecule We have not investigated this in further detail

5.7 Conclusions

The cyclodextrin derivatives described in this chapter were designed to detect neutral organic guest molecules, like cyclohexanol and adamantanecarboxylic acid, by fluorescence spectroscopy At neutral pH, they are not sensitive towards guest molecules due to a strong self-inclusion of the fluorophore attached to the cyclodextrin By lowering the pH we have succeeded in reaching Sf-values that are comparable with values displayed by compounds described in the literature One of the compounds is able to detect concentrations of adamantanecarboxylic acid as low as 5 x 10^{7} M, a value that has never been reached using other cyclodextrin based sensor molecules Although our compounds have the disadvantage that they only show high responses at low pH, their stability in aqueous solutions is much higher which allows the use of these compounds in other detection formats like sol-gel matrices The design of novel sensor systems clearly is difficult and the following points have to be considered The fluorophore should be able to form a self-inclusion complex with the CD-unit The linking spacer must have a well-defined length and such a rigidity that the self-inclusion process is not favoured too much For future applications it may be of interest to test more hydrophilic polarity probes which can make the self-inclusion a more balanced process

5.8 Experimental

General For general experimental details on syntheses see Section 2 6
Compounds **15** and **18** were prepared as described in Chapters 2 and 3, respectively Eluents used in chromatography were mixtures (v/v) of ethyl acetate, ethanol and water (A (100 4 2), B (100 8 4), C (100 14 8), D (100 30 16), E (100 2 1)) 5-Dimethylamino-1-naphthalenesulphonyl chloride (dansyl chloride) was used as received

Fluorescence measurements: Fluorescence measurements were performed using a Perkin-Elmer luminescence spectrometer LS50B Solutions were measured in a 1 00 cm (4 ml) or a 0 50 cm (2 ml) quartz cuvette, which was placed in a thermostatted (25 0 ±0 1 °C) holder All measurements were performed using an aqueous solution of KH_2PO_4 (0 05 M) and citric acid (0 05 M) which was adjusted to the required pH by addition of NaOH Stock solutions of the host molecule had concentrations of 1 x 10^{5} or 1 x 10^{6} Mol l^{-1}, depending on the experiment To portions of these stock solutions, guest was added to give concentrations of 6 x 10^{5} M for adamantanecarboxylic acid (ACA) and 6 x 10^{3} M for cyclohexanol (CH) Higher concentrations of ACA could not be obtained due to the limited solubility of this probe The excitation wavelengths were 305 nm at pH 1 and 333 nm at pH 7 The excitation slits were set at 2 5 nm and the emission slits at 2 5-10 nm, depending on the concentration of the probe under investigation No oxygen quenching of the fluorescence was observed The titrations were carried out by starting with a solution of the host molecule (850 µl), followed by a gradual

increase of the guest concentration by adding portions (at least 10) of the guest-stock solution (5-1000 µl) After every addition a new spectrum was recorded and stored in a computer By subtracting the first from the last spectrum a differential spectrum was obtained, which revealed the wavelength at which the maximum changes in fluorescence intensity took place At this wavelength the fluorescence intensity was determined for all spectra This intensity was plotted as a function of the guest concentration Subsequently these data were fitted assuming the formation of a 1 1 host-guest complex, as described in Section 4 2 All measurements were performed in duplo

Fluorescence lifetimes: Fluorescent lifetimes were measured with picosecond time correlated single photon counting using a Hamamatsu micro channel plate (R3809) detector, employing a frequency doubled DCM dye laser which was synchronously pumped with a mode locked Argon ion laser resulting in 317 nm 19 ps FWHM pulses For a more detailed description of the experimental set up see reference 37

1,1,1-Triphenyl-10-amino-2,5,8-trioxadecane (21)

Ethanol (250 ml) was saturated with ammonia gas at 0 °C for 30 min After addition of 1 0 g (1 83 mmol) of compound **20** (which was prepared as described in Chapter 4) the reaction mixture was transferred to an autoclave and heated for 15 h at 80 °C The reaction mixture was concentrated *in vacuo* and the residue dissolved in ethyl acetate The solution was washed with water and brine and dried over $MgSO_4$ After removal of the solvent, compound **21** was obtained as a light orange oil which was >95% pure according to ^{1}H-NMR Yield 512 mg (72%) ^{1}H-NMR ($CDCl_3$) 7 5-7 1 (m, 15H, *H*-Ar), 4 5 (s, 2H, NH_2), 3 7-3 5 (m, 8H, CH_2-O) 3 2 (t, 2H, CH_2-O-C$(CH_3)_3$)

1,1,1-Triphenyl-10-(5'-dimethylamino-1'-naphtalenesulphonamido)-2,5,8-trioxadecane (22)

To a solution of 492 mg (1 26 mmol) of compound **21** in 50 ml of THF and 0 2 ml of triethylamine (1 1 equiv) was added 380 mg (1 1 equiv) of dansyl chloride This mixture was stirred for 6 h and concentrated *in vacuo* The residue was dissolved in ethyl acetate and the solution washed with water and brine and dried over $MgSO_4$ After removal of the solvent the crude product was purified by column chromatography (silica 60, 40g, ethyl acetate/ hexane 1/9, v/v) which yielded compound **22** as a yellow viscous oil Yield 389 mg (49%) ^{1}H-NMR ($CDCl_3$) 8 6-8 1, 7 5-7 4 and 7 3-7 1 (3xm, 21H, *H*-Ar), 3 7-3 0 (m, 12H, CH_2-O) 2 8 (s, 6H, CH_3-N) EI-MS (m/e) 624 (M+)

8-(5'-Dimethylamino-1'-naphtalenesulphonamido)-3,6-dioxaoctanol (23)

Compound **22** (352 mg, 0 56 mmol) was dissolved in 25 ml of dichloromethane and 1 6 g (20 equiv) of $ZnCl_2$ was added Dry methanol was added to obtain a homogeneous system After 20 h stirring at room temperature, the reaction mixture was concentrated *in vacuo* and the residue dissolved in 50 ml of ethyl acetate The resulting solution was washed with water and brine, dried over $MgSO_4$, filtered and rotary evaporated The crude product was purified by column chromatography (silica 60, 15g, ethyl acetate/hexane, 2/1, v/v) yielding compound **23** as a yellow oil Yield 171 mg (72%) ^{1}H-NMR ($CDCl_3$) 8 6-8 1, 7 7-7 4 and 7 3-7 1 (m, 6H, *H*-Ar), 3 8-3 4 (m, 12H, CH_2-O), 3 15 (t, 2H, CH_2-amide), 2 8 (s, 6H, CH_3-N), 1 7 (br s, 2H, N*H* and O*H*) EI-MS (m/e) 382 (M+)

8-(5'-Dimethylamino-1'-naphtalenesulphonamido)-1-tosyloxy-3,6-dioxaoctane (14)

A mixture of 148 mg of **23** (0.44 mmol) and 96 mg of tosyl chloride (1.1 equiv.) in 25 ml of pyridine was stirred for 20 h. Another portion of tosyl chloride (50 mg) was added and the reaction mixture stirred for an additional 18 h. After concentrating the reaction mixture *in vacuo* the residue was dissolved in 50 ml of dichloromethane and the solution was washed with 1M HCl, a saturated solution of $NaHCO_3$, and brine. After drying over $MgSO_4$, an evaporation of the solvent, the crude product was purified by column chromatography which afforded a yellow oil. Yield 159 mg: (66%). EI-MS (m/e): 536 (M+). ^{1}H-NMR ($CDCl_3$) : 8.6-8.1, 7.-7.1 (m, 10H, *H*-Ar), 4.1 (t, 2H, CH_2-O-Tos), 3.6 (t, 2H, CH_2CH_2-O-Tos), 3.5-3.4 (m, 6H, CH_2-O), 3.1 (t, 2H, CH_2-amide), 2.8 (s, 6H, CH_3-N), 2.3 (s, 3H, CH_3).

Mono-3-deoxy-3-(5'-dimethylamino-1'-naphtalenesulphonamido)heptakis(6-O-*tert*-butyldimethylsilyl)-β-cyclodextrin (17)

Compound **15**, 203 mg (0.092 mmol), was dried (60 °C, 0.5 mm Hg, 1 h) and dissolved in 15 ml of dry THF. To this solution was added 23.6 mg (0.95 equiv.) of dansyl chloride and 0.02 ml of triethyl amine at 0 °C. After 10 h the reaction mixture was allowed to warm to room temperature and stirred for an additional 24 h. The reaction mixture was concentrated *in vacuo*, the residue dissolved in 50 ml of ethyl acetate, and the solution washed with a saturated solution of $NaHCO_3$ (twice), brine and dried over $MgSO_4$. After removal of the solvent *in vacuo*, the crude product was chromatographed (30 g silica, eluent A) to yield compound **17** as a slightly yellow solid. Yield 127 mg: (59% calculated from dansyl chloride). This compound was directly desilylated and only characterised by FAB-MS. FAB-MS (m/e): 2182 (M+Na).

Mono-3-deoxy-3-(5'-dimethylamino-1'-naphtalenesulphonamido)-β-cyclodextrin (12)

Compound **19**, 121 mg (0.056 mmol) was dried (60 °C, 1 h, 0.1 mm Hg) and dissolved in 20 ml of THF. After addition of 0.5 ml of a stock solution (1M) of TBAF (8.9 equiv.) in THF, the reaction mixture was refluxed for 24 h. After concentration *in vacuo*, the residue was dissolved in a minimum amount of ethanol/water (2/1, v/v) and the product precipitated by addition of ethyl acetate. The precipitate was collected by centrifugation. Further purification was achieved by exclusion chromatography (Fractogel TSK HW-40 (F) (Merck), bed volume 200 ml, flow rate 13.2 ml per h, eluent water). After lyophilisation, the compound was obtained as a white solid. Yield 48 mg: (63%). Mp: 270 °C (dec.). ^{1}H-NMR (D_2O) : 8.48, 8.39, 8.26 (3xd, 3H, *H*-Ar), 7.71 and 7.67 (2xt, 2x1H, *H*-Ar), 7.37 (d, 1H, *H*-Ar), 5.02, 4.98, 4.96 (3xd, J=3.6 Hz, 5H, *H*-1), 4.70 (d, J=6.1 Hz, 1H, *H*-1), 4.4 (br. s, 1H, *H*-1), 3.88-3.40 (m, 48H, *H*-2, *H*-3, *H*-4, *H*-5 and *H*-6), 2.84 (s, 6H, CH_3-N). ^{13}C-NMR (D_2O) : 152.6, 136.02, 131.35, 130.79, 130.10, 129.56, 125.83, 121.91, 117.78 (*C*-Ar), 105.00-102.38 (*C*-1), 82.71-79.83, 76.90-71.07 (*C*-2, *C*-3 *C*-4 and *C*-5), 61.67-59.34 (*C*-6), 46.71 (CH_3-N). FAB-MS (m/e): 1360 (M+1). Anal. Calcd for $C_{54}H_{75}O_{36}N_2S.7H_2O$: C, 43.64; H, 6.04; N, 1.88; S, 2.16. Found : C, 43.14; H, 6.00; N, 1.94; S, 2.27.

Mono-2-O-(2'-(5"-dimethylamino-1"-naphtalenesulphonamido)ethyl)heptakis (6-O-*tert*-butyldimethylsilyl)-β-cyclodextrin (19)

To a solution of 408 mg of dried (0.21 mmol, 60 °C, 0.1 mm Hg, 1 h) compound **18** (which was prepared as described in Chapter 3) in 25 ml of dry THF was added 52.8 mg (0.95 equiv.) of dansyl chloride and 0.3 ml of triethyl amine. After 18 h stirring at room temperature, the reaction mixture was concentrated *in vacuo*, the residue dissolved in 50 ml of ethyl acetate, and the solution washed with a aqueous saturated solution of $NaHCO_3$ (twice), brine and dried over $MgSO_4$. After removal of the solvent *in vacuo*, the crude product was chromatographed (60 g silica, eluent E) to yield pure compound **19** as a white solid. Yield 263 mg: (58% calculated from **16**). Mp: >300 °C. ^{1}H-NMR ($CDCl_3/CD_3OD$, 5/1, v/v) : 8.53, 8.33, 8.21 and 7.20 (4xd, 4x1H, *H*-Ar), 7.60 and 7.53 (2xt, 2x1H, *H*-Ar), 4.94 (m, 7H, *H*-1), 3.99-3.48 (m, 44H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6 and CH_2CH_2-O), 3.14 (t, 2H, CH_2-NH), 2.89 (s, 6H, CH_3-N), 1.04-0.73 (s, 63H, CH_3-*t*-Butyl), 0.08-0.00 (s, 42H, CH_3-Si). ^{13}C-NMR ($CDCl_3/CD_3OD$, 5/1, v/v) : 151.51, 135.05, 130.03, 129.69, 129,45, 128.72, 128.07, 122.97, 119.09 and 115.06 (10x*C*-Ar), 102.39-102.04 and 99.43 (*C*-1), 81.66-80.59, 73.24-71.83 (*C*-2, *C*-3 *C*-4 and *C*-5), 70.93 (CH_2CH_2-O), 61.54 (*C*-6), 45.16 (CH_3-N), 42.62 (*C*-NH), 25.61-25.49 (CH_3-*t*-Butyl), 18.05 (*C*-$(CH_3)_3$), -5.38-(-5.49) (CH_3-Si). FAB-MS (m/e): 2232 (M+Na). Anal. Calcd for $C_{98}H_{184}O_{37}N_2SSi_7.2H_2O$: C, 52.33; H, 8.37; N, 1.25; S, 1.42. Found : C, 52.07; H, 8.22; N, 1.21; S, 1.50.

Mono-2-O-(2'-(5"-dimethylamino-1"-naphtalenesulphonamido)ethyl)-β-cyclodextrin (13)

To a solution of 252 mg (0.114 mmol) of dried (60 °C, 1 h, 0.1 mm Hg) compound **19** in 20 ml of THF was added a stock solution (1M, 1.0 ml) of TBAF (8.5 equiv.) in THF. The reaction mixture was refluxed for 20 h. Water (5 ml) was added and the reaction mixture was concentrated *in vacuo*, the residue dissolved in minimum amount of ethanol/water (2/1, v/v) and the product precipitated by addition of ethyl acetate. The precipitate was collected by centrifugation. TBA-salts were removed by cation exchange chromatography using an ion exchange column (Dowex) in the NH_4^+-form and water as the eluent. The compound was obtained as a white solid by lyophilisation of the product containing fractions. Yield 118 mg: (76%). Mp: 280 °C (dec.). ^{1}H-NMR (DMSO-d6) : 8.46, 8.27, 8.10 and 7.25 (4xd, 4x1H, *H*-Ar), 7.64 and 7.60 (2xt, 2x1H, *H*-Ar), 4.89 and 4.81 (d+s, 1H+6H, *H*-1), 3.64-3.44 and 3.17-3.04 (2xm, 44H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6 and CH_2CH_2-O), 2.97 (t, 2H, CH_2-NH), 2.82 (s, 6H, CH_3-N). ^{13}C-NMR (DMSO-d6) : 151.38, 135.84, 129.49, 129.04 128.24, 128.04, 123.71, 119.03 and 115.12 (*C*-Ar), 101.95-101.71 and 99.78 (*C*-1), 81.82-80.82, 73.03-71.60 (*C*-2, *C*-3 *C*-4 and *C*-5), 69.97 (CH_2CH_2-O), 59.91-57.52 (*C*-6), 45.13 (CH_3-N), 42.30 (*C*-NH). FAB-MS (m/e): 1412 (M+1). Anal. Calcd for $C_{56}H_{79}O_{37}N_2S.5H_2O$: C, 44.86; H, 5.94; N, 1.87; S, 2.14. Found : C, 44.56; H, 6.12; N, 1.88; S, 1.98.

Mono-2-O-(8'-(5"-dimethylamino-1"-naphtalenesulphonamido)-3',6'-dioxaoctyl)heptakis(6-O-*tert*-butyldimethylsilyl)-β-cyclodextrin (26)

To a solution of 485 mg (0.25 mmol) of dried (100 °C, 0.5 mm Hg, 5 h) compound **25** in 25 ml of dry THF was added NaH (60% dispersion in mineral oil, 25 mg, 2.5 equiv). The solution was stirred for at least 17 h at room temperature and one h at reflux temperature. Subsequently 73.5 mg (0.56 equiv) of compound **14** was added. After 24 h refluxing, the reaction mixture was concentrated *in vacuo*, the residue dissolved in ethyl acetate and the

resulting solution washed with water, with brine and dried over $MgSO_4$ After removal of the solvent *in vacuo*, the crude product was chromatographed (100 g silica, eluent B) to yield pure compound **26** as a light yellow solid Yield 104 mg (33% calculated from **14**) Mp >300 °C ^{1}H-NMR ($CDCl_3/CD_3OD$, 7/1, v/v) 8 54, 8 34, 8 23 and 7 21 (4xd, 4x1H, *H*-Ar), 7 52 (m, 2H, *H*-Ar), 5 00 and 4 94 (d and s, 6H+1H, *H*-1), 4 01-3 38 (m, 58H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6 and C*H*$_2$-O), 3 14 (t, 2H, C*H*$_2$-NH), 2 90 (s, 6H, C*H*$_3$-N), 1 04-0 73 (s, 63H, C*H*$_3$-*t*-Butyl), 0 08-0 00 (s, 42H, C*H*$_3$-Si) ^{13}C-NMR ($CDCl_3/CD_3OD$, 7/1, v/v) 151 5, 135 15, 129 93, 129 59, 129,39, 128 73, 127 09, 122 97, 118 92 and 114 97 (10x*C*-Ar), 101 98-101 69 and 100 35 (*C*-1), 81 93-80 08, 73 17-71 96 (*C*-2, *C*-3 *C*-4 and *C*-5), 71 14-69 05 (*C*H$_2$-O), 61 68-61 29 (*C*-6), 45 06 (*C*H$_3$-N), 42 37 (*C*-NH), 25 53-25 40 (*C*H$_3$-*t*-Butyl), 17 98 (*C*-(CH$_3$)$_3$), -5 52 (*C*H$_3$-Si) FAB MS (m/e) 2322 (M+Na) Anal Calcd for $C_{102}H_{192}O_{39}N_2SSi_7$ $1H_2O$ C, 52 90, H, 8 38, N, 1 21, S, 1 38 Found C, 52 88, H, 8 76, N, 1 24, S, 1 10

Mono-2-O-(8'-(5"-dimethylamino-1"-naphtalenesulphonamido)-3',6'-dioxaoctyl)-β-cyclodextrin (14)

To a solution of 80 mg (0 035 mmol) of dried compound **26** (40 °C, 1 h, 0 5 mm Hg) in 15 ml of THF was added 0 30 ml of a stock solution (1M) of TBAF in THF, and the reaction mixture was refluxed for 18 h After concentration *in vacuo*, the residue was dissolved in minimum amount of ethanol/water (2/1, v/v) and the product precipitated by addition of ethyl acetate The precipitate was collected by centrifugation Further purification was achieved by cation exchange chromatography using an ion exchange column (Dowex) in the NH_4^+-form and water as the eluent The compound was obtained as a white solid by lyophilisation of the product-containing fractions Yield 41 mg (79%) Mp >300 °C ^{1}H-NMR (D_2O) 8 57, 8 36, 8 30 and 7 32 (4xd, 4x1H, *H*-Ar), 7 78 and 7 75 (2xt, 2x1H, *H*-Ar), 5 11 and 4 98-4 95 (d and m, 1H+6H, *H*-1), 3 84-3 35 (m, 54H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6 and C*H*$_2$-O), 3 13 (br t, 2H, C*H*$_2$-NH), 2 85 (s, 6H, C*H*$_3$-N) ^{13}C-NMR (D_2O) 152 40, 135 82, 131 00, 130 59, 130 42, 130 30, 125 75, 120 72, 116 60 (*C*-Ar), 103 33-102 98 and 101 79 (*C*-1), 83 23-81 59, 74 64-72 19 (*C*-2, *C*-3 *C*-4 and *C*-5), 70 90-69 69 (*C*H$_2$-O), 61 50-61 18 (*C*-6), 46 90 (*C*H$_3$-N), 43 75 (*C*H$_2$-NH) FAB-MS (m/e) 1499 (M+1) Anal Calcd for $C_{60}H_{94}O_{39}N_2S$ $5H_2O$ C, 45 34, H, 6 55, N, 1 76, S, 2 02 Found C, 45 37, H, 6 29, N, 1 88, S, 2 22

Methyl-3-deoxy-3-(5'-dimethylamino-1'-naphtalenesulphonamido)-α-D-altropyranoside (27)

This compound was prepared starting from methyl-2,3-anhydro-4,6-O-benzylidene-α-D-mannopyranoside, which was a kind gift of Dr Gordon Chittenden The opening of this epoxide with ammonia was performed in an analogous way as described by Myers et al [42] to give methyl-3-amino-3-deoxy-4,6-O-benzylidene-α-D-altropyranoside as a crystalline product This compound reacted directly with dansyl chloride as follows 196 mg was dissolved in 25 ml of THF and 0 1 ml of triethylamine was added together with 176 mg of dansyl chloride at 0 °C The reaction mixture was stirred 6 h at 0 °C, followed by one day at room temperature After removal of the organic solvent *in vacuo*, the residu was dissolved in ethyl acetate and the solution washed with a saturated solution of $NaHCO_3$ and with brine The organic layer was dried over $MgSO_4$ and concentrated Further purification was achieved by column chromatography (silica 60, 50 g, eluent 3% MeOH in $CHCl_3$) yielding 265 mg (74%) of the dansylated product as a yellow glass This compound (110 mg) was directly deprotected by

dissolving it in 50 ml of aqueous 0.01 M H_2SO_4 and heating at 80 °C for 4 h. Purification of the product was achieved by washing the acidic water layer with chloroform to remove the starting material and the benzaldehyde. After neutralisation of the water layer with $NaHCO_3$, the product was obtained by extraction with chloroform. After evaporation of the dried ($MgSO_4$) organic layers, the product **27** was obtained in pure form. Yield 53 mg (58%) ^{1}H-NMR 90 MHz ($CDCl_3$:CD_3OD, 5:1, v:v) : 8.7-8.15 and 7.7-7.1 (2xm, 2x3H, *H*-Ar), 4.4 (s, 1H, *H*-1), 3.95-3.3 (m, 7H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6), 3.35 (s, 3H, O-C*H*$_3$), 2.9 (s, 6H, C*H*$_3$-N). EI-MS (m/e): 426 (M+). Anal. Calcd for $C_{19}H_{26}O_7N_2S$: C, 53.51; H, 6.14; N, 6.57; S, 7.52. Found : C, 53.50; H, 6.28; N, 6.23; S, 6.97.

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CHAPTER 6

Detection of Organic Vapours in Air using Cyclodextrin-Coated Piezoelectric Crystals

6.1 Introduction

Piezoelectric crystals are of great potential value for the development of new types of gas sensors which can be used to detect gaseous compounds in research and industry. In order to make these inert crystals respond to gas molecules they must be coated with a layer that interacts with the gas. Ideally, such a layer should contain receptor molecules that selectively recognise the molecules of the analyte. Cyclodextrin derivatives may be suitable for this purpose, because they have been applied in gas chromatography, as the stationary phase, to achieve the separation of various organic compounds, including stereoisomers.[1] In view of this we decided to investigate CD derivatives as coating materials for piezoelectric crystals with the ultimate goal of constructing gas sensors that respond rapidly and in a highly selective way. Preliminary results will be described in this chapter.

6.1.1 Theory of the quartz crystal microbalance detection technique

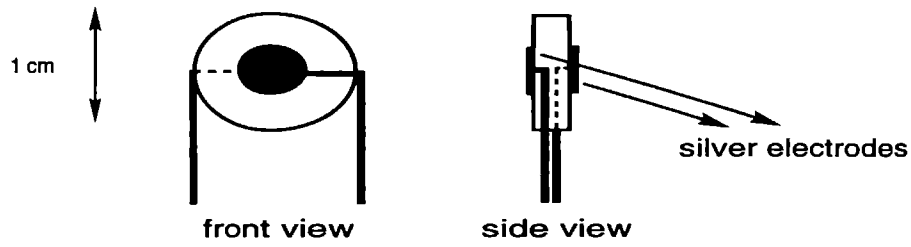


Figure 6.1 *Schematic drawing of a piezoquartz crystal*

Figure 6.1 shows a schematic drawing of a piezoelectric quartz crystal. It is made from a single quartz crystal that has been cut at specific angles to the principal optical axis. On both sides of the crystal two silver (or gold) electrodes are affixed. If an alternating potential difference is applied between these two electrodes (typically 5 V), the crystal starts to vibrate at its oscillation

frequency (typically 9-12 MHz). This resonance frequency strongly depends on the mass of the crystal and can be determined accurately (0.1-1Hz) using commercially available frequency counters. This type of crystals, therefore, can be used to measure very small amounts of compounds, as was already described by Mayer et al. in 1957.[2] Two years later Sauerbrey derived an expression relating the change in frequency to the mass of material deposited on the crystal.[3] This expression (1) is shown in a simplified form below

$$\Delta F = \frac{-2.3 \times 10^{6} \times F_0^2 \times \Delta m}{A} \qquad (1)$$

In formula (1) ΔF is the frequency change observed (Hz), F_0 the initial frequency of the crystal (MHz), A is the surface area of the coating (cm^2) and Δm the change in the mass of the crystal (g). From equation (1) it follows that with a quartz microbalance(QMB)-device resonating at 9 MHz, frequency variations of approximately 1Hz per 10^{-9} g can be obtained which allows the detection of nanogram quantities. The Sauerbrey-equation is valid for mass changes on a quartz crystal that do not change the viscoelastic properties of the resonating mass. In practical applications this requirement is not always met, leading to deviations from equation (1).[4, 5]

6.1.2 Literature examples

To make a QMB-crystal suitable for the detection of gas molecules it should be covered with a coating that interacts with the gas-molecule of interest. The use of a coated QMB-crystal as a gas sensor was described for the first time in 1964.[6] Several chromatographic absorbents were applied as the chemically sensitive coating which allowed the detection of vapours like benzene, toluene and water. Since then, many researchers have looked for better and more selective coatings. For example a hydrogen chloride sensor was constructed that could detect HCl-gas at concentrations down to 0.001 ppm within 30 seconds using a coating of triphenylamine [7] Many other substances like aromatic compounds, ammonia, explosives and nerve gasses have been measured with the help of a great variety of chemical coatings [8, 9, 10,11]

The application of supramolecular compounds as coating materials in QMB-sensors is a logical step in the development of more selective responses, since a large variety of compounds is available nowadays which have been thoroughly studied with respect to their abilities to form inclusion complexes.[12] Göpel et al. [13] have investigated CDs, a crown ether, a calix[4]arene and a hexalactam (a host molecule based on three phenyl groups connected via six amide bonds) as coating materials for QMB-crystals. This latter derivative showed a response of 65 Hz upon exposure to 90 ppm of trichloroethene in air while the other macrocyclic compounds

(including α-CD and β-CD) displayed smaller responses. Permethylation of β-cyclodextrin yielded a sensor that responded to 0.1 % trichloroethene in air. The α-CD analogue gave no response at all probably because its cavity was too small to encapsulate trichloroethene.[14] Good guest molecules for CDs in aqueous solution, like toluene and benzene, were not tested by Göpel. These molecules, however, can be efficiently detected in another system in which the modified CD-derivatives 2,6-per-O-(*t*-butyldimethylsilyl)-α-CD and 2,6-per-O-methyl-β-CD are applied as coatings. Using the α-CD derivative a five times higher response was observed for benzene than for toluene.[15]

The use of organised multilayers as coatings for QMB-crystals is relatively new. Okahata et al. used piezoelectric quartz crystals coated with an immobilised synthetic multibilayer film.[16, 17, 18, 19, 20] This coating was prepared from the surfactant dioctadecyldimethylammonium bromide ($2C_{18}N^+2C_1Br^-$) and sodium poly(styrenesulfonate) (PSS^-Na^+), which were both dissolved in $CHCl_3$ and deposited on a QMB-crystal to form lamellar structures of lipid bilayers.[16] These coated crystals were used to detect bitter substances like strychnine at very low concentrations in aqueous media: 19 ppm of the compound resulted in a frequency change of 500 Hz. The chemical structure of the building blocks forming the multilayer is an important factor in determining the selectivity of the sensing device. For example, linear long chain alcohols can penetrate more deeply into the bilayer of surfactants containing lineair alkyl chains than branched and sterically bulky alcohols, leading to lower responses for the latter molecules. Another example is the response towards cholesterol of a multibilayer coating which was built up from cholesterol derivatives. This response was much higher than that of a lipid multilayer prepared from $2C_{18}N^+2C_1Br^-$ and PSS^-Na^+.[18] These examples show that multilayers can be used for the selective recognition of guest molecules. The above mentioned cholesterol multibilayer was also used for the selective detection of CDs in aqueous media. The addition of α-CD, which is too small to encapsulate a cholesterol unit, to the system gave no frequency change of the QMB. When, however, either β or γ-CD was added a decrease of the frequency of the QMB was observed indicating an interaction with the multilayer. Desorption of γ-CD by placing the QMB in clean water was reversible but the desorption of β-CD resulted in a solvation of the multilayer, probably as a result of the formation of a very strong, soluble, inclusion complex between cholesterol and β-CD.[19]

Many substances can be detected in aqueous solutions with the above mentioned multibilayer systems [20] but also detection in the gas phase has been reported, e.g. odorants and perfumes.[17] The driving force for the detection is the lipophilic character of the analytes resulting in a direct adsorption to the lipid multilayer matrix. A similar process may be involved in olfactory reception which was suggested to begin with non-specific lipophilic interactions.[21]

Non-covalent interactions between sulfur atoms and a gold surface can be used to prepare monolayers on a QMB-crystal.[22] The spontaneous formation of monolayers from alkanethiol and thiophenol on gold-coated QMB-crystals has been reported.[23,9,24] Also di-*n*-decyl sulfide and calixarenes, modified with long sulfur containing alkyl chains have been shown to form self-organised monolayers on a gold-plated QMB-crystal.[4] These monolayers were used for the detection of perchloroethylene. The calixarene monolayer showed higher frequency changes toward this analyte than the di-alkyl sulfide monolayer. This result was ascribed to the presence of the cavitand molecules in the former layer which acted as additional recognition sites.

To develop devices that may respond selectively to guest molecules of interest we designed the system depicted in Figure 6.2, which consists of a monolayer of amphiphilic molecules containing CDs as the additional recognition sites. The formation of such a monolayer and its successive transfer onto a crystal is in principle possible using Langmuir-Blodgett techniques.[25]

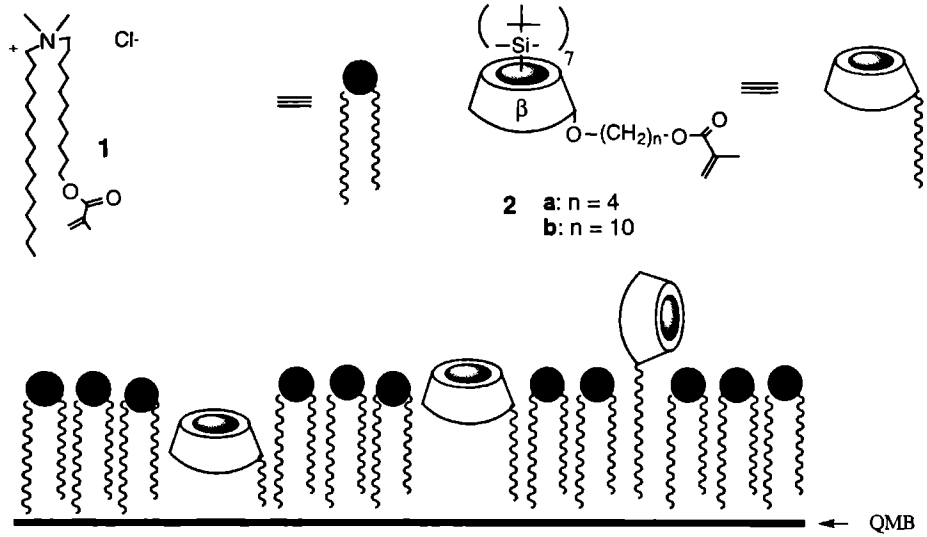
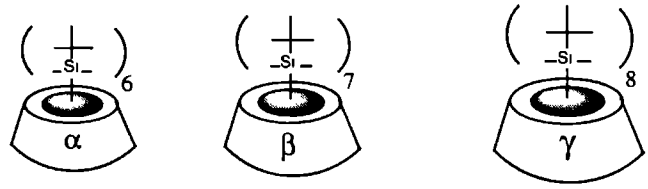


Figure 6.2 *Lipid monolayer with recognition sites*

The amphiphilic molecule **1** (Figure 6.2) has already been used by our group and by others to synthesise polymerised bilayers.[37, 27] Its use will eventually lead to more stable coatings. In order to introduce extra recognition sites we designed molecules **2a** and **2b** which can be copolymerised with the amphiphilic matrix. By varying the length of the polymerisable side chain in **2** we can change the position of the CD ring in the monolayer. The presence or absence of the protecting silyl groups in compounds **2** can also be used to control the location of the CD rings. The silylated compounds will be located in the inner part of the monolayer, whereas the

desilylated compounds will be situated at the interface. The construction of a system as shown in Figure 6.2 is quite difficult and also the frequency changes of a QMB-crystal covered with only one monolayer will be very small (1-65 Hz).[13,4] Furthermore, the accuracy of a QMB-sensory device is very sensitive to external influences like moisture, flow-rate, experimental set-up, vapour concentration and temperature.[28, 10] Preliminary experiments showed that our QMB device operated within an experimental error of approximately 50 Hz. For this reason, a system as shown in Figure 6.2 is not easily realised in our laboratory. We nevertheless decided to embark on this project using the available experimental set-up to see whether it would be justified to do more advanced studies in the near future with more sophisticated and sensitive equipment. First, we tested the silylated CDs **3-5** for their ability to detect organic vapours and, more importantly, for their ability to do this in a selective way.



3 **4** **5**

Second, we investigated whether CD-derivatives like compounds **2** could be polymerised and whether the polymerisation would have an effect on the binding properties of the host molecules. In particular we were interested to see if cooperative binding of a guest molecule by two or more CD-rings would take place. The latter feature can be important if large molecules like androstenone have to be detected in the gas phase. The detection of this steroid and other related molecules is of interest for the development of a boar taint sensor for the meat industry.[29] This chapter deals with a preliminary study towards the applicability of the detection system described above.

6.2 Coating materials

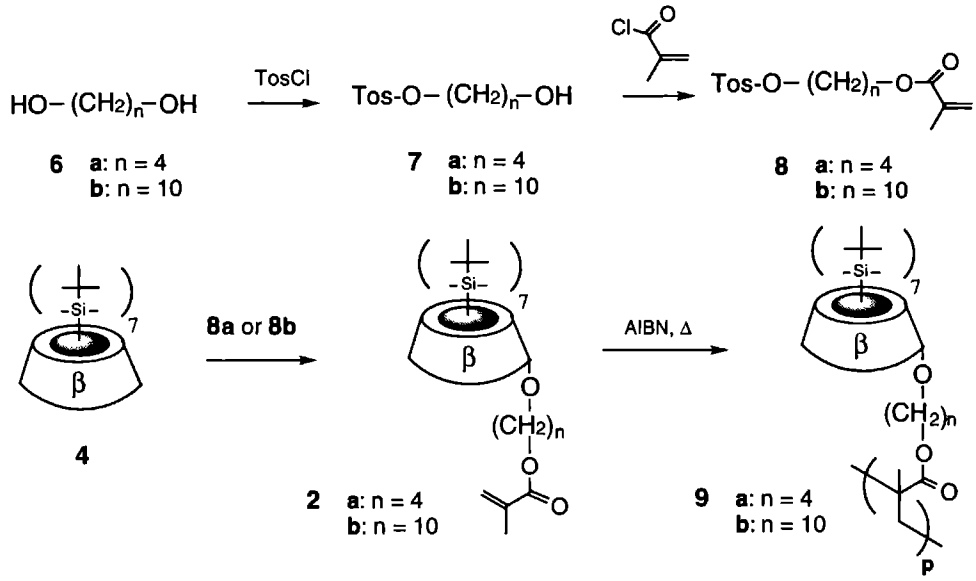
6.2.1 Synthesis of silylated cyclodextrins

The syntheses of compounds **3** and **4** have already been described in Chapter 2. Compound **5** was prepared in an analogous way by reaction of γ-CD with 10.3 equivalents of *tert*-butyldimethylsilyl chloride. For solubility reasons pyridine was used as the solvent instead of THF. Compound **5** could be obtained in 61% yield after repeated column chromatography. The ^{1}H NMR-spectrum of this compound in $CDCl_3$ did not show the symmetrical signals

anticipated on the basis of the 8-fold symmetry (C_8-symmetry) present in the molecule Since compound **5** was pure according to FAB-MS, TLC, and elemental analysis, the unexpected complexity of the NMR spectrum is proposed to be due to the presence of two or more conformers which interconvert slowly on the NMR time-scale This was investigated by dissolving the compound in a different solvent, *viz* a mixture of $CDCl_3$ and CD_3OD (5 1, v v) and recording a new ^1H NMR spectrum (results not shown) This spectrum was in agreement with a symmetrical structure Since the NMR spectrum of **5** in $CDCl_3$ revealed that the signal of one of the silyl protecting groups was shifted downfield with respect to the other signals, it is reasonable to assume that one glucose moiety is twisted in such a way that its silyl protecting group is different from the others and possibly included intramolecularly in the cavity These observations are in accordance with results obtained by Lehn et al [30] who showed that symmetrically substituted β-CD-derivatives can undergo a slow exchange between conformers having C_7- and C_1-symmetry, as the result of self-inclusion of one of the substituents

6.2.2 Synthesis and characterisation of polymerised β-cyclodextrin derivatives

Several randomly polymerised CDs have been described in the literature [31] These polymers were obtained by reaction of CDs with epichlorohydrin[32] or with diepoxides [33] and also by reaction of CD derivatives with poly(allylamine) [34 35] Harada et al have synthesised CD polymers with a more well-defined structure which were obtained by polymerisation of mono-acryloyl-β-CD derivatives [36] These monofunctionalised β-CDs were prepared by an acyl transfer reaction and purified by gel chromatography A disadvantage of acryloyl-β-CDs is their instability towards hydrolysis in basic aqueous media because the polymerisable function is connected to the CD via an ester linkage We expected that an ether linkage instead of an ester linkage and a methacryloyl function instead of an acryloyl function would lead to more stable CD polymers, and therefore decided to synthese the two polymerisable CD-derivatives **2** as is shown in Scheme 6 1 The synthesis started with the monotosylation of the diols **6a** and **6b** The resulting monotosylated compounds **7** were purified by column chromatography and reacted with methacryloyl chloride in the presence of triethylamine as a base to give compounds **8** As was already mentioned in Chapter 3, the deprotonation of compound **4** at its C-2 hydroxyl group leads to an alkoxy anion which can react with alkyl tosylates, but not with alkyl chlorides or alkyl iodides Reaction of compounds **8a** and **8b** with the deprotonated molecule **4** yielded the corresponding compounds **2a** and **2b**, in 17 and 23 %, respectively These compounds were characterised by NMR, FAB-MS and elemental analyses The polymerisation of compound **2a** was effected by reaction with AIBN (7 mol%) in refluxing 2-butanone This



Scheme 6.1

yielded compound **9a**. At first the occurrence of polymerisation was not recognised since the R_f-values of the starting material and the product, surprisingly, happended to be similar on TLC. Attempts to precipitate the product using various solvents were ineffective, which further supported the impression that no polymerisation had taken place. The ^1H NMR spectrum of the reaction mixture, however, revealed that the vinylic protons had disappeared, which is indicative of polymerisation. To characterise the reaction products they were desilylated by refluxing with 8.8 equivalents of TBAF in THF for 12 h. The resulting water-soluble product **10a** (see Fig. 6.3) was purified by exclusion chromatography (Fractogel TSK HW-40 (F), Merck), which yielded two fractions. The first fraction eluted with a volume of 25-35 ml (void volume of the column) which according to the specifications of the column indicated a molecular weight larger than approximately 10.000. The second fraction eluted at 47 ml which probably corresponds to a molecular weight of approximately 3000-9000. To investigate the molecular weight distribution in more detail, the polymer mixture was applied to a PLgel exclusion column, which was connected to a refractive index (RI) detector followed by a multi angle laser light scattering (MALLS) detector. The signals of these detectors were used to determine the weight % (RI) and the weight average molecular weight (RI and MALLS) of every fraction. From the shape of the chromatograms the polydispersity (defined as the quotient of the weight average molecular weight and the number average molecular weight) of the fractions could be calculated. The results obtained from this experiment are summarised in Table 6.1.

Table 6.1 *Gel permeation chromatography data of polymer 10a* [a]

Fraction number	Elution volume (ml)	Poly-dispersity	Weight % [b]	10^{-3} x $\overline{M}_w$ [c]	Number of CD-units [c]
1	17	1.1	50	7.6 ± 1 3	6 ± 1
2	16	1.1	27	17 ± 3	13 ± 2
3	13.5	> 3	15	93 ± 25	73 ± 20
4	12	> 2.6	5	800 ± 125	630 ± 100
5	10	> 1.3	3	160 ± 70	120 ± 50

[a] Obtained using a PLgel column, attached to a refractive index detector followed by a multi angle laser light scattering detector, eluent DMSO H_2O, 9 1 v v [b] Estimated error 10%
[c] Errors in the given values are estimated from the signal/noise ratio of the detected signals

An interesting conclusion which can be derived from Table 6 1, is the absence in the polymerisation mixture of fragments with a molecular weight between 7.6 x 10^3 and 17 x 10^3 An explanation for this observation may be that initially a fast polymerisation reaction takes place, up to six monomeric units, followed by a decrease of the rate of the polymerisation due to steric congestion. After a while mainly hexamers are present in the reaction mixture, which can dimerise to yield a fraction that consists of approximately 12 monomeric units It is also possible that two hexamers associate to give a non-covalent complex The technique used cannot distinguish between these two possibilities The higher masses observed (more than 73 monomeric units) are probably the result of higher aggregates of the hexamers or dodecamers This can be concluded from the eluent volume which is not in accordance with the observed masses of the last three fractions and also from the large polydispersities of these fractions

The fraction containing the hexamer was obtained in pure form and used to perform binding studies with the water-soluble porphyrin TsPP (see Figure 6 3) Titration experiments were carried out (Figure 6.3) and the observed changes in fluorescence intensity were analysed using formula (2) in which the symbols are defined as explained in Chapter 4 and $[H]_t$ is the oligomer concentration.

$$[HG] = \frac{([H]_t + [G]_0 + K_b^{-1}) - \{([H]_t + [G]_0 + K_b^{-1})^2 - 4 . [H]_t . [G]_0\}^{1/2}}{2} \quad (2)$$

The latter parameter can be described by:

$$[H]_t = \frac{[\text{ monomeric units }]}{p} \quad (3)$$

In equation (3) p represents the number of CD-units per oligomer. The changes in fluorescence intensity as a function of the oligomer concentration were fitted using K_b and p as the varying parameters.

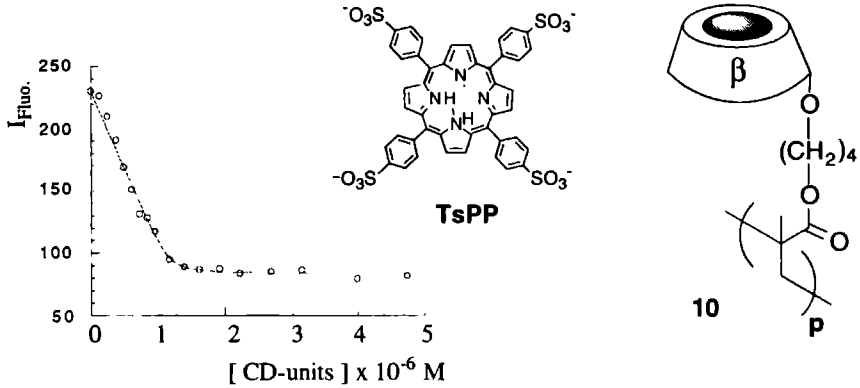


Figure 6.3 *Titration curve of TsPP (2.0 x 10^{-7} M, 0.1 M phosphate buffer, pH 7.0) with oligomer **10a** (fraction 1 of Table 6.1)*

The best fit of the data points was obtained with values of p = 5.8 ± 0.3 and K_b = (4 ± 2) x 10^8 M^{-1}, the correlation coefficient being r = 0.9968 (line drawn through data points in Figure 6.3). The length of the oligomer calculated from this titration curve is in excellent agreement with the value (6 ± 1) determined by the laser diffraction method. The very high binding constant indicates a very efficient binding of the porphyrin TsPP in the hexamer. The exact binding geometry of the complex (e.g. how many CD-units are involved in the binding) can not be deduced from the titration data. Fits assuming that two or three TsPP molecules are bound per oligomer (with equal K_b's) did not give good correlation coefficients (r < 0.98). We therefore conclude that the binding stoichiometry is probably of a 1:1 (hexamer:TsPP) complex.

6.3 Detection of organic compounds in air using cyclodextrins

To study the interaction of gas molecules with coated piezo quartz crystals we used a set up as is schematically represented in Figure 6.4. It contains two modes: one for detection (Figure 6.4 right) and another one for cleaning the system (Figure 6.4 left). The switching between these two modes can be achieved by turning a six-way valve (not shown for reasons of clarity). In a typical experiment a crystal coated with a silylated CD-derivative was mounted in the sensing unit. The system was kept in the cleaning mode until a constant signal was observed. In this way also the frequency change as a result of the coating itself (ΔF_{coat}) could be determined

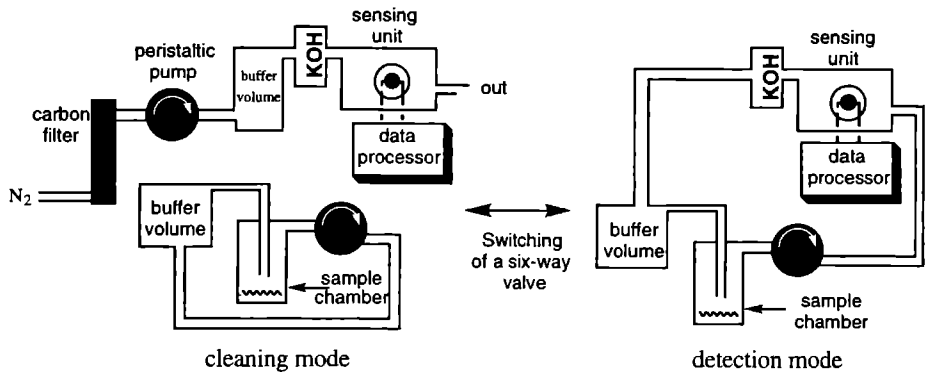


Figure 6.4 *Set-up used for the detection of organic vapours*

provided the frequency of the uncoated crystal was known. After flushing the whole system with nitrogen gas for approx. 30 minutes, the evaporation chamber was filled with the compound to be detected and the system was kept in the cleaning mode until saturation of the nitrogen atmosphere (2 l) with the compound was reached. To start an experiment, the six-way valve was switched and the frequency changes as the result of absorption of the analyte were monitored and stored in a computer. In Figure 6.5 typical curves obtained for ethanol and toluene using a crystal coated with compound **4** are presented.

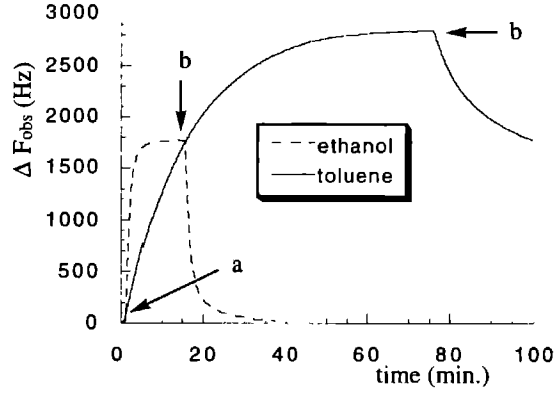


Figure 6.5 *Response curves of a sensor, coated with compound 4, to N_2 saturated with ethanol or toluene vapour. The valve was switched to the measuring mode at arrow (a) and switched back to fresh N_2 at arrow (b).*

From these curves it can be concluded that both compounds show an interaction with the CD-matrix resulting in high frequency changes (ΔF_{obs}). The adsorption of toluene onto the crystal

is slower than that of ethanol, which might be a result of the larger size of the toluene molecule which makes its penetration in the CD-matrix slower. The desorption of toluene was also slower than that of ethanol, which could be due to the stronger interactions between the toluene molecules and the CD-matrix. An important factor to be controlled in the system is the humidity since variations in the water content of the vapour can lead to frequency changes up to 500 Hz. The air used in the system, therefore, was dried over KOH (see Figure 6.4). To test the influence of the temperature on the response of the crystal, experiments were carried out at different temperatures of the sensing unit (the temperature of the evaporation room was thermostatically controlled at 25 °C). The results are shown in Figure 6.6.

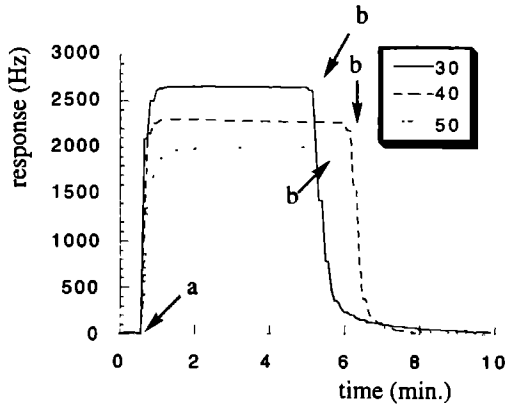


Figure 6.6 *Response curves of a sensor, coated with compound 4, to N_2 saturated with ethanol at different temperatures of the detection chamber. The valve was switched to the measuring mode at arrow (a) and switched back to fresh N_2 at arrow (b).*

It can be seen that even at 50 °C very good responses are obtained for ethanol. This is of interest for future applications since the gas sensors based on lipid multilayers reported in the literature, showed a large decrease of the response above 45 °C.[17]

It is very difficult to coat a crystal with a stock solution and a syringe in a reproducable manner. The frequency changes due to the coating (ΔF_{coat}) of two crystals are not always the same and therefore the responses of these crystals to a saturated ethanol vapour in air can be different. This is probably the result of the mass distribution of the coating on the crystal, which is not homogeneous. It has been reported in the literature that mass changes at the center of the crystal result in a larger decrease of the frequency than mass changes at the side of the crystal.[3] For our experiments we used coated crystals with rather similar responses after coating (ΔF_{coat}). To be able to compare the responses of two crystals with different CD-coatings to several organic compounds, we introduced a corrected response frequency ($\Delta F_{c,mw}$). This $\Delta F_{c,mw}$ is the value

of ΔF_{obs} standardised to the average response of a coating with a ΔF_{coat}-value of 25000 Hz. Since compound **3** contains more cavities per gram than **4** we also introduced a factor that corrects the frequency change for the number of cavities in the coating relative to coating **4** (see equation 4).

$$\Delta F_{c,mw} = \Delta F_{obs} \times \frac{25000}{\Delta F_{coat}} \times \frac{Mw_{coating}}{1934\ g.mol^{-1}} \qquad (4)$$

In this formula $Mw_{coating}$ is the molecular weight of the coating molecules and 1934 $g.mol^{-1}$ is the molecular weight of coating **4**. In Figure 6.7 the corrected frequency changes for three different crystals, coated with **3**, **4** and **5**, upon exposure to toluene vapour are presented as a function of time.

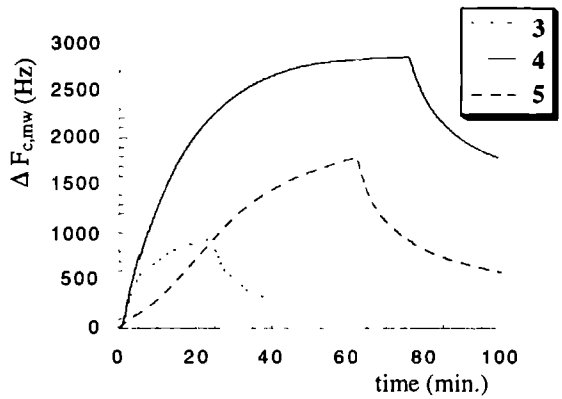


Figure 6.7 *Detection of toluene vapour with crystals coated with 3, 4 and 5.*

It can be seen that the best response to toluene vapour is obtained with a coating containing the β-CD derivative **4**. The host molecule **3** apparently is too small to form a stable complex with toluene, resulting in a smaller frequency change. Molecule **5** has a large cavity and therefore may be expected to bind more than one toluene molecule and to give a larger frequency change than molecule **4**. This is not the case, as can be seen in Figure 6.7. The reason for this deviating behaviour probably is the following. Compound **5** has a cavity that is filled by one of its silyl groups in solution (see Section 6.2.1). If this self-inclusion also is present in the solid state, the γ-CD ring has to undergo a conformational change before a toluene molecule can enter its cavity, which leads to a smaller respons. This process may be reflected in the beginning (0-20 min.) of the response curve of **5**, which clearly is different from the curves observed for the coatings **3** and **4**.

The system described in Figure 6.4 was found to have several disadvantages. It was difficult to clean when one type of vapour had to be changed for another. The system also leaked due to the multitude of connecting parts. The flow rates (30-50 ml.hr^{-1}) which could be obtained with the peristaltic pump were too small to achieve a fast loading of the crystal and also made regeneration of the crystal a time consuming process (especially for toluene). We therefore designed the following system (Figure 6.8) using a commercially available suction pump that allowed a flow rate of 350 ml.hr^{-1}.

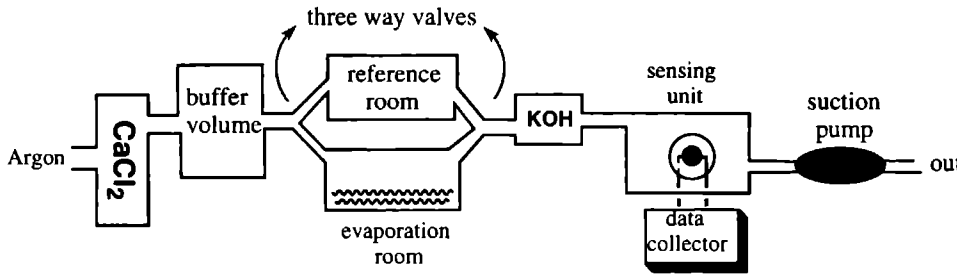


Figure 6.8 *Improved set-up for the detection of organic vapours.*

A possible disadvantage of this system is that no saturation of the vapour will be reached if the vapour pressure of the compound to be detected, is too low. We found, however, responses for ethanol that were even higher than the values measured with the system of Figure 6.4, indicating that the vapour pressure of this compound is high enough to reach saturation in an argon atmosphere (see Figure 6.9). An advantage of the system of Figure 6.8 is the very small detection chamber (2.3 cm^3) in comparison with the closed system of Figure 6.4 (46 cm^3). This, in combination with the higher flow rate,[28,26] enhances the adsorption and desorption rate of the vapour, as can be seen in Figure 6.9.

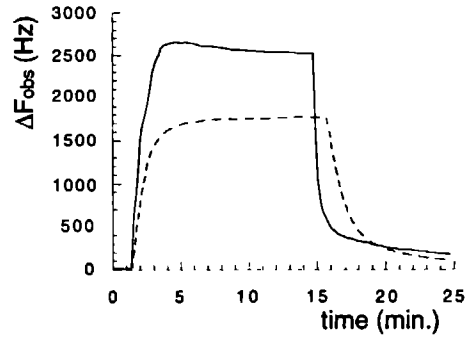


Figure 6.9 *Response curves to ethanol vapour measured with the systems described in Figure 6.4 (dotted line) and in Figure 6.8 (solid line)*

With the open system we studied the response of several coatings towards vapours of compounds that differed in structure and vapour pressure. The responses after 40 minutes were determined. The results are collected in Table 6.2.

Table 6.2 *Corrected values of the frequency changes of coated crystals after exposure to vapours of various organic compounds*

Compound	Vapour pressure (mm Hg) [a]	$\Delta F_{c,mw}$ (Hz)		
		Coating **3**	Coating **4**	Coating **5**
ethanol	60	1700	2600	2200
m-xylene	10	3150	>5000 [b]	4550
methyl-cyclohexane	40	2450	>5000 [b]	4000
α-pinene	5	750	1800	2450 [c]
cyclohexanol	1	1500 [c]	2600 [c]	2750 [c]
skatol	<< 1	-	250	-

[a] Taken from ref. 38. [b] Higher frequency changes could not be detected due to an irreversible change (of the coating) of the crystal [c] No saturation of the crystal was observed even after 40 min

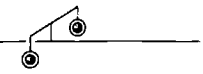
The γ-CD coating **5** always showed a slower response to guest molecules than the other two coatings as was already observed for toluene vapour For the interpretation of the results summarised in Table 6 2, we assume that the vapour pressure is an indication for the concentration of the guest molecules in air. As can be seen the best responses are observed for *m*-xylene and methylcyclohexane, although the response of coating **4** is irreversible due to a process which is not yet understood α-Pinene has been reported to form more stable complexes with β-CD than with α-CD in a gas-liquid chromatographic system [39] This difference is also reflected in the frequency changes measured for the silylated CD-derivatives **3** and **4**. The bulky α-pinene molecule is even better bound in coating **5** (Table 6 2) These observations indicate that complexation of the analyte *in* the cavity of the cyclodextrins does play an important role in the molecular recognition process in the gas phase Cyclohexanol can be detected more accurately with coatings **4** and **5** than with coating **3**, although the differences in frequency are smaller than those observed for α-pinene. This is probably due to the fact that cyclohexanol is less bulky than α-pinene and fits better in all three host molecules It is surprising, however, that this compound can be detected with such high responses since the vapour pressure of cyclohexanol is much smaller than the vapour pressures of the other compounds tested. The alcohol functionality probably contributes to the interaction with the coating material due to the formation of hydrogen bonds.

From the frequency change measured for the combination *m*-xylene and **4** it can be calculated that in every β-CD-cavity at least four guest molecules are bound, which is very unlikely Two other possibilities, therefore, have to be considered which may cause the high frequency changes The first possibility is that guest molecules are also located between the coating molecules Since the *m*-xylene vapour is detected with some selectivity, this can not be the only explanation The second possibility is that the binding of *m*-xylene molecules leads to changes in the viscoelastic properties of the coating [5] These changes will result in deviations from the Sauerbrey equation (1) which in turn can lead to more sensitive responses than expected on the basis of this equation [4] Since the Sauerbrey equation may not be valid in our case, no conclusions can be drawn without further experiments

A special feature of the crystals coated with **4** is their ability to detect skatol, which is one of the compounds held responsible for boar taint [29] Since the concentration of this solid compound in air is very low (based on its vapour pressure) a frequency change of 250 Hz (Table 6 2) means that this compound has a very good interaction with the CD-matrix The structurally related compound indole is known to be included in β-CD in aqueous solution [40] It is likely that in the gas phase such an inclusion also is possible and the complex with skatol might be stabilised by the formation of a hydrogen bond This sensitive detection of skatol is promising for industrial applications of the type of sensors described in this chapter

6.4 Concluding remarks

In this chapter we have investigated the detection of vapours of various organic compounds in air with the help of cyclodextrin derivatives A correct fit between the vapour molecules and the CDs in the coating layer appears to be important for a good response The formation of hydrogen bonds between the guest molecules and the coating material is favourable because it leads to a more stable complex and a higher response The obtained selectivities with the coatings tested are not large enough to assure the selective detection of a compound in a mixture of other compounds in air Since the curves describing the development of the frequency change in time depend on the vapour that is detected, it must be possible to use this extra information for the assignment of the components in a complex mixture using a neural network Such a network has been described in the literature to identify five different whiskies with a recognition probability of 76% using a set of eight QMB-sensors which were coated with different materials [41] To study the interaction of guest molecules with our CD-derivatives in more detail it would be of interest to prepare a monolayer of these molecules on a QMB in such a way that all the CD-cavities are pointing in the direction of the gas phase Since the frequency changes that result from guest binding in a monolayer will be very small, it may be better to use the more sensitive surface acoustic wave (SAW) detector in these studies [9] This sensing device



can operate at much higher frequencies (430 MHz) because the surface rather than the whole SAW-crystal is brought into resonance. Since the Sauerbrey equation is also valid for this type of device, the frequency changes upon guest binding will be much larger than with a QMB-device. Recently, Dickert et al. [42] have described a sensing system based on a SAW-device in which a modified CD was anchored to a monolayer by an aliphatic spacer. This system allowed the detection of 10 ppm of tetrachloroethylene in air, showing the full advantage of an SAW-based sensor when compared to a QMB sensor.
Very recently, cyclodextrin derivative **4** was used to prepare a stable monolayer at the air-water interface.[43] Using Langmuir-Blodgett techniques it must be possible to deposit monolayers of this compound on a SAW-device. Another way in which cyclodextrins may be organised in the form of a monolayer is by using gold-sulfur interactions, as was recently described by Kaifer and co-workers.[44] These authors synthesised β-CDs which were per-thiolated at the C-6 positions. When these compounds were deposited on a gold surface they formed self-assembled (although distorted) monolayers. Both the Langmuir-Blodgett technique and the use of sulfur-gold interactions are promising possibilities to prepare new sensor devices.

We have shown that the polymerisation of compound **2a** is feasible and leads to well-defined hexameric structures. The hexamer shows a very high affinity for a water soluble porphyrin. This makes a study of the use of oligomeric cyclodextrins for the detection of (ditopic) molecules in the gas phase and the aqueous phase very worthwile.

Although a monolayer matrix consisting of CD-derivatives and amphiphilic molecules has not yet been realised, the preliminary results presented in this chapter show that all ingredients for a sensor device based on such a matrix are available.

6.5 Experimental

General: For general experimental details on syntheses see Section 2.6. Tosyl chloride was recrystallised from hexane before use. Eluents used in chromatography were mixtures (v/v) of ethyl acetate, ethanol and water (A: (100:2:1); B: (100:4:2); C: (100:14:8)) or a mixture (v/v) of n-propanol : ethylacetaat : water : ammonia (D: (5:3:3:1)). 2,2'-Azobis(2-methylpropionitrile) (AIBN) was used as received and stored at 4°C.

QMB-experiments: The piezoelectric crystals were of the AT-cut quartz type (HC-50, CL=30pF, 9 MHz) with silver-plated electrodes on both sides (Klove Electronics, Heerhugowaard). The crystal was brought into resonance by a commercially available power supply set at 5 V d.c., which also monitored the frequency of the vibrating crystal at the same time (fragrance sensor, Toyo Corp. type SF-105P). The data monitored by the frequency counter were collected on a recorder (Yokogawa Hokushin Electric, type YEW 3087) coupled

to a computer that stored the data (time, temperature, frequency). In the closed system of Figure 6.4 two peristaltic pumps were used (Ismatec, type mp-ge), while in the open system of Figure 6.8 a suction pump was used which was part of the suction measurement kit of a fragrance sensor.

Preparation of the coatings: Stock solutions of 24 mg of silylated CDs in 5 ml of dichloromethane were used for the coating of the crystals. Using a syringe 4.5 µl of this stock solution was deposited on each side of the crystal. A typical coating mass was approximately 40 µg which resulted in a frequency change (ΔF_{coat}) of (25-30) x 10^3 Hz. The crystals used in the system of Figure 6.8 were coated on one side only and at the center of the crystal (coating mass approx. 25 µg, frequency change approx. 25 x 10^3 Hz).

Octakis(6-O-*tert*-butyldimethylsilyl)-γ-CD (5)

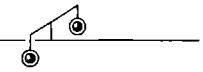
γ–Cyclodextrin was dried (100 °C, 1.7 mm Hg, 8 h) and 6.83 g of this compound was dissolved in 100 ml of pyridine. At 0 °C 8.2 g (10.3 eq) of *tert*-butyldimethylsilyl chloride in 50 ml of pyridine was added in 0.5 h. After stirring for 5 days at room temperature the reaction mixture was poured in 1 l of ice/water and stirred for 15 min.. The white precipitate was filtered off (using Celite) and dissolved in dichloromethane (150 ml), washed twice with aqueous HCl (1M), once with 100 ml of a saturated $NaHCO_3$ solution and once with brine. The resulting organic layer was dried ($MgSO_4$) and concentrated *in vacuo* yielding the crude product. Repeated (three times) column chromatography (1.5 kg of silica, eluent B) yielded compound **5** as a white solid. Yield: 6.86 g (61% yield). Mp: 289 °C (dec.) (lit.[45] 268-270, dec.) R_f(C)= 0.25. ^{1}H-NMR ($CDCl_3$:CD_3OD, 5:1, v:v) : 4.86 (s, 8H, *H*-1), 3.85-3.23 (m, 48H, *H*-2, *H*-3, *H*-4, *H*-5 and *H*-6), 0.74 (m, 72H, C*H*$_3$-C), -0.11 (m, 48H, C*H*$_3$-Si). ^{13}C-NMR (CD_3Cl:CD_3OD, 5:1, v:v): 101.86 (*C*-1), 80.26, 73.19, 72.93 and 72.41 (*C*-2, *C*-3, *C*-4 and*C*-5), 61.61 (*C*-6), 25.60 (*C*H_3-C), 18.03 (CH_3-*C*), -5.43 (*C*H_3-Si). FAB-MS (m/e): 2233 (M+Na). Anal. Calcd for $C_{96}H_{192}O_{40}Si_8.H_2O$: C, 51.72; H, 8.77. Found : C, 51.56; H, 8.71.

4-Hydroxybutyl-*p*-toluenesulfonate (7a)

Sodium hydride (60% dispersion in mineral oil, 2.4 g, 60 mmol) was rinsed with hexane to remove the oil. The cleaned NaH was added to a solution of 1,4-butanediol (26.2 g, 260 mmol) in 50 ml of THF. After gas evolution had stopped, tosyl chloride was added (10.9 g, 57 mmol) at 0 °C. After stirring the reaction mixture for 1 h, the THF was removed *in vacuo* and the residue dissolved in 100 ml of diethyl ether. The solution was washed with a saturated solution of $NaHCO_3$ and with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by column chromatography (silica 60, 200 g, eluent: ethylacetate:hexane, 1:1 followed by 1:2, v:v) yielding compound **7a** as a colourless oil. Yield: 7.8 g (57% based on tosyl chloride). ^{1}H-NMR ($CDCl_3$) : 7.79 and 7.34 (2xd, 2x2H, Ar-*H*), 4.06 (t, 2H, TosO-C*H*$_2$), 3.61 (t, 2H, HOC*H*$_2$), 2.45 (s, 3H, Ar-C*H*$_3$), 1.66-1.51 (m, 4H, C*H*$_2$). EI-MS (m/e): 244 (M+).

10-Hydroxydecyl-*p*-toluenesulfonate (7b)

To a solution of 1,10-decanediol (9.9 g, 57 mmol) in 100 ml of pyridine was added at 0 °C 4.21 g (22 mmol) of tosyl chloride. After stirring the reaction mixture for one day, the solvent was removed *in vacuo* and the residue dissolved in 500 ml of ethyl acetate. The solution was



washed with 1M HCl to remove the pyridine, washed with a saturated solution of $NaHCO_3$ and with brine, dried over $MgSO_4$ and concentrated *in vacuo* The crude product was purified by column chromatography (silica 60, 250 g, eluent ethylacetate hexane, 1 1, v v) yielding compound **7a** as a slightly yellow oil Yield 1 2 g (17% based on tosyl chloride) ^{1}H-NMR ($CDCl_3$) 7 8 and 7 3 (2xd, 2x2H, Ar-*H*), 4 0 (t, 2H, TosO-C*H*$_2$), 3 5 (t, 2H, HOC*H*$_2$), 2 4 (s, 3H, Ar-C*H*$_3$), 1 8-0 9 (m, 16H, C*H*$_2$)

4-Methacryloxybutyl-*p*-toluenesulfonate (8a)

To a solution of 6 0 g of compound **7a** in 40 ml of dichloromethane was added 3 3 ml of pyridine and 4 4 g of freshly distilled methacryloyl chloride at 0 °C The reaction mixture was stirred overnight at room temperature After addition of 100 ml of dichloromethane the reaction mixture was washed with HCl (1 M), water and brine, dried over $MgSO_4$ and concentrated *in vacuo* Purification of the crude product was achieved by column chromatography (silica, 20 g, eluent $CHCl_3$ MeOH, 9 1, v v) yielding compound **8a** as a colourless oil Yield 5 6 g (73% yield) ^{1}H-NMR ($CDCl_3$) 7 7 and 7 2 (2xd, 2x2H, Ar-*H*), 6 0 (s, 1H, C=C*H*), 5 5 (s, 1H, C=C*H*), 4 2-3 9 (m, 4H, OC*H*$_2$), 2 4 (s, 3H, Ar-C*H*$_3$), 1 9 (s, 3H, C=C-C*H*$_3$), 1 8-1 6 (m, 4H, C*H*$_2$) EI-MS (m/e) 312 (M+)

10-Methacryloxydecyl-*p*-toluenesulfonate (8b)

This compound was prepared as described for compound **8a** using 1 2 g of **7b**, 0 5 ml of pyridine and 0 65 g (1 7 equiv) of freshly distilled methacryloyl chloride Purification of the crude product was achieved by column chromatography (silica 60, 40 g, eluent $CHCl_3$ MeOH, 9 1, v v) yielding compound **8b** as a colourless oil Yield 650 mg (45% yield) ^{1}H-NMR ($CDCl_3$) 7 8 and 7 3 (2xd, 2x2H, Ar-*H*), 6 0 (s, 1H, C=C*H*), 5 5 (s, 1H, C=C*H*), 4 3 3 9 (m, 4H, OC*H*$_2$), 2 4 (s, 3H, Ar-C*H*$_3$), 2 2-1 0 (m, 19H, C*H*$_2$ and C=C-C*H*$_3$) EI-MS (m/e) 396 (M+)

Mono-2-O-(4-methacryloxybutyl)-heptakis(6-O-*tert*-butyldimethylsilyl)-β-CD (2a)

Silylated cyclodextrin **4** (7 59 g) which was dried (105 °C, 0 6 mm Hg, 7h) before reaction, was dissolved in 250 ml of dry THF A 60% dispersion of NaH in mineral oil (480 mg, 3 equiv) was added and the solution was stirred for one night at room temperature The temperature of the solution was raised to reflux temperature and 1 17 g (1 5 equiv) of compound **8a** was added in two portions (with a 24 h interval) After refluxing for four days, the reaction mixture was concentrated *in vacuo*, the residue dissolved in dichloromethane and the solution washed with a saturated $NaHCO_3$-solution and with brine (twice), dried ($MgSO_4$) and concentrated The crude product was purified by column chromatography (500 g silica, eluent B) yielding compound **2a** as a white solid Yield 1 35 g (17%) R_f(C)= 0 5 IR 1715 and 1635 cm^{-1} (CO and C=C) Mp 259 °C FAB-MS (m/e) 2098 (M+Na) ^{1}H-NMR ($CDCl_3$ CD_3OD, 1 1, v v) 5 92 (s, 1H, C=C*H*), 5 40 (s, 1H, C=C*H*), 4 76 (m, 7H, *H*-1), 3 99-3 05 (m, 46H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6 and OC*H*$_2$), 1 75 (s, 3H, C=C-C*H*$_3$), 1 55 (m, 4H, C*H*$_2$), 0 70 (s, 63H, C-C*H*$_3$), -0 15 (m, 42H, Si-C*H*$_3$) ^{13}C-NMR ($CDCl_3$ CD_3OD, 1 1, v v) 167 70 (*C*O), 135 97 (C=*C*-CO), 125 39 (C=*C*H$_2$), 102 00 (*C*-1), 81 26-80 57, 73 16-72 82, 72 23-72 02 (*C*-2,*C*-3,*C*-4,*C*-5 and *C*H$_2$-OC(O)), 64 24 (*C*H$_2$-O), 61 49 (*C*-6), 25 48 (*C*H$_3$-C), 24 68 and 25 92 (CH$_2$-spacer), 17 82 (C_{quart}-CH$_3$), -5 58 (*C*H$_3$-Si) Anal Calcd for $C_{92}H_{180}O_{37}Si_7$ C, 53 25, H, 8 74 Found C, 52 94, H, 8 75

Mono-2-O-(10-methacryloxydecyl)-heptakis(6-O-*tert*-butyldimethylsilyl)-β-CD (2b)

Dried (100 °C, 0 4 mm Hg, 8 h) silylated cyclodextrin **4** (1 33 g) was dissolved in 50 ml of dry THF A 60% dispersion of NaH in mineral oil (100 mg, 3 6 equiv) was cleaned by washing with hexane and added to the solution of **4** This reaction mixture was stirred for 3 h at reflux temperature and 270 mg (1 0 equiv) of compound **8a** was added in three portions (after 0, 24 and 36 h) After three days refluxing, the reaction mixture was concentrated *in vacuo*, dissolved in ethyl acetate and the solution washed with a saturated $NaHCO_3$-solution and with brine (twice), dried ($MgSO_4$) and concentrated The crude product was purified by column chromatography (100 g silica, eluent A) yielding compound **2b** as a slightly yellow solid Yield 340 mg (23%) R_f(C)= 0 6 IR 1720 and 1635 cm $^{-1}$ (CO and C=C) Mp 270 °C FAB-MS (m/e) 2182 (M+Na) ^{1}H-NMR ($CDCl_3$ CD_3OD, 1 1, v v) 6 07 (s, 1H, C=C*H*), 5 55 (s, 1H, C=C*H*), 4 93 (m, 7H, *H*-1), 4 12-3 19 (m, 46H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6 and OCH_2), 1 90 (s, 3H, C=C-CH_3), 1 62 (m, 4H, CH_2), 1 27 (m, 12, CH_2), 0 86 (s, 63H, C-CH_3), -0 05 (m, 42H, Si-CH_3) ^{13}C-NMR ($CDCl_3$ CD_3OD, 1 1, v v) 168 57 (*C*O), 136 84 (C=*C*-CO), 125 90 (C=*C*H_2), 102 80 (*C*-1), 81 99-72 74 (*C*-2, *C*-3, *C*-4, *C*-5), 65 47 (CH_2-O), 62 28 (*C*-6), 30 10-28 99, 26 54 and 26 09 (CH_2-spacer), 26 20 (CH_3-C), 18 68 (C_{quart}-CH_3), -4 86 (CH_3-Si) Anal Calcd for $C_{98}H_{192}O_{37}Si_7$ H_2O C, 54 06, H, 8 98 Found C, 53 98, H, 8 95 Polymerisation of this compound has not been investigated yet

Polymerisation of compound 2a and deprotection of the polymer 10a

Polymerisation of the monomer was performed under an argon atmosphere All solutions were degassed thoroughly before use Compound **2a**, 500 mg (0 24 mmol) was dissolved in 10 ml of 2-butanone and 2 8 mg (7 mol%) AIBN was added After stirring the reaction mixture for 24 h at reflux temperature, the crude product was isolated by evaporating the solvent R_f(C)= 0 5 ^{1}H NMR revealed the disappearance of the vinyl protons of compound **2a** Polymer **9a** was directly desilylated to **10a** by addition of 30 ml of THF and 2 ml of a 1 M solution of TBAF in THF (8 5 equiv) After refluxing for 12 h the reaction mixture was concentrated *in vacuo* and the residue dissolved in 0 5 ml of ethanol Addition of acetone (analytical grade) led to the precipitation of the polymer which was collected by centrifugation In this way compound **10a** was obtained as a white solid Overall yield 162 mg (53%) R_f(D)= 0-0 1 The polymer fraction containing the smallest molecular weight could be isolated by exclusion chromatography (Fractogel HW-40 S, bed volume 200 ml, eluent water, flow rate 14 4 ml h^{-1}, UV-detection at 226 nm) The fractions with higher molecular weight polymer were not fully separated, and eluted at volumes between 25-39 ml, whereas the fraction with the lower molecular weight component eluted at 47 ml A further characterisation of the molecular weight distribution was achieved using two PLgel columns, mixed A and mixed E (30 cm x 4 mm) which were connected in series The polymer fractions (220 µl of a 1% weight solution of the polymer in DMSO/water, 90/10, v/v) were separated using these columns The detection was achieved with a refractive index (RI) detector which was connected in series with a multi angle laser light scattering (MALLS) detector (Wyatt) The eluent was 90% DMSO/10% water (v/v) The assumption was made that the temperature dependence of the refractive index was equal to the values of amylose, dextrans and pullulanes, dn/dT = 0 074 Integration of the RI-signal was used to determine the mass of every fraction The molecular weight can be determined from the quotient of both detector signals

The hexameric fraction was further characterised Mp 285 °C (dec) ^{1}H-NMR (D_2O) 5 14 (d, 2H, *H*-1), 5 02 (m, 12H, *H*-1), 3 91-3 51 (2 x m, approx 88H, *H*-2, *H*-3, *H*-4, *H*-5, *H*-6 and OCH_2), 1 6 (m, approx 4H, CH_2), 1 2 (br m, C-CH_3) ^{13}C-NMR (D_2O) 103 13 and 101 49 (*C*-1), 82 85-81 52, 74,39-72 96 (*C*-2, *C*-3, *C*-4, *C*-5), 62 57 and 61 40 (CH_2-O and *C*-6), 28 95, 26 76 (CH_2-spacer) IR 1650 cm^{-1} (C=O) Solubility in H_2O > 360 g l^{-1}

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Summary

Cyclodextrins (CDs) are a group of naturally occurring cyclic oligomers of glucose which result from the enzymatic degradation of starch There are three major products of the degradation reaction α-, β- and γ-cyclodextrin which contain 6, 7 and 8 glucose units, respectively The molecules are basket-shaped and have a well-defined hydrophobic cavity which can be used to encapsulate (apolar) guest molecules in aqueous media The hydroxyl groups located at both rims of the cyclodextrin ring make the CDs very soluble in water

The aim of the research described in this thesis is the development of new supramolecular systems based on monofunctionalised cyclodextrins To obtain the latter compounds, the cyclodextrins were first protected at their primary side with *tert*-butyldimethyl silyl groups, as is described in *Chapter 2* This made the cyclodextrin derivatives soluble in organic solvents and allowed their synthesis and purification on a larger scale One of the remaining unprotected hydroxyl groups, present in the silylated cyclodextrins, could be converted into an amino group yielding a mono-functionalised cyclodextrin Two of these molecules were covalently linked via different spacer molecules to give cyclodextrin homo-dimers By using two cyclodextrins of different size (α-, and β-cyclodextrin), a cyclodextrin hetero-dimer could be obtained NMR experiments showed that if the alkyl spacer is sufficiently long (eight methylene units), it can be encapsulated in one of the two CD cavities

From previous investigations performed in our group, it is known that manganese(III) porphyrins are excellent catalysts for the epoxidation of alkenes with molecular oxygen The reduction of the manganese(III)porphyrin which is required for the reaction to proceed, can be achieved by sodium formate using a rhodium-bipyridine complex as a co-catalyst In *Chapter 3* the synthesis of two cyclodextrin dimers which contain a third binding place in the spacer moiety, are described Two cavities of these dimers can be used to encapsulate the porphyrin catalyst and the third binding site to immobilise a substrate (alkene) or the co-catalyst One of the two cyclodextrin dimers had a linking spacer derived from diphenylglycoluril, which can bind dihydroxybenzene guest molecules For the synthesis of this dimer a silylated cyclodextrin was mono-functionalised with α,α'-dibromoxylene which was subsequently coupled to the diphenylglycoluril based spacer molecule Although the compound was formed, it could not be isolated in pure form using chromatographic methods Therefore this compound was not further investigated as a catalyst The second cyclodextrin dimer containing a bipyridine spacer, however, could be purified and was used to immobilise a rhodium metal centre The resulting rhodium complex was capable of reducing water-soluble manganese porphyrins With this rhodium CD dimer, and the manganese porphyrin as catalytic system, oxygen activation and epoxidation of nerol were achieved in aqueous

solution. The turnover numbers for this reaction, however, were too low to make this system interesting for industrial applications.

To get a better insight in the binding geometry and the stoichiometry of complexes between porphyrins and cyclodextrin dimers, a binding study was undertaken which is described in *Chapter 4*. Dimers which were linked via flexible alkyl spacers could form two types of complexes with the porphyrins, viz. syn-complexes in which two adjacent phenyl groups of the porphyrin are encapsulated and anti-complexes in which the encapsulation involves two opposite phenyl groups. Short spacers resulted in the formation of syn-complexes, whereas a longer spacer gave a mixture of both syn- and anti-complexes, as could be concluded from ^{1}H-NMR data. The bipyridine linked dimer gave an unusual 2:2 complex in which two dimers encapsulate two porphyrins in an anti-fashion. The formation of this complex was facilitated by the addition of zinc(II) ions.

Chapter 4 also describes the binding of three anilinonaphthalenesulphonates in cyclodextrin homo- and hetero-dimers. These asymmetrical guest molecules could be encapsulated in cyclodextrin hetero-dimers in a site-specific way: the naphthyl part of the guest molecule in the β-cyclodextrin ring and the smaller anilino part in the α-cyclodextrin ring. This site-specific binding might be used in the future development of catalysts for regioselective reactions.

Chapter 5 describes the synthesis of three dansyl modified cyclodextrins which differ in the length of the spacer that links this fluorophore to the cyclodextrin moiety. The dansyl group of these compounds can be included in the CD cavities which makes these compounds suitable as sensor molecules since the complexation of an analyte will result in the displacement of the fluorophore from the cavity. This, in turn, will lead to a decrease of the fluorescence intensity of the dansyl group. Using this principle organic compounds like adamantanecarboxylic acid could be detected. Due to the strong self-inclusion of the dansyl group, however, the changes in the fluorescence intensity were very small. Lowering of the pH led to the protonation of the dansyl moiety and thereby to a weakening of the self-inclusion process. This gave rise to much higher responses upon the addition of adamantanecarboxylic acid which made detection of very low concentrations of this guest molecule possible.

In *Chapter 6* deals with silylated cyclodextrins as gas sensing materials in a sensing device that is based on the quartz microbalance detection technique. The latter technique makes use of a quartz crystal resonating at a well-defined frequency which is dependant on the mass of the crystal. By coating this crystal with silylated cyclodextrin derivatives, a device was obtained which showed a response to vapours of organic compounds. From the responses of the tested compounds it was concluded that the size of the silylated cyclodextrin (α-, β- or γ-cyclodextrin) has to match the size of the gas molecule for an optimal effect. It was also concluded, however, that a large fraction of the gas molecules probably is not located in the cavities of the host molecules but in between these molecules. Interestingly, the molecules skatol and cyclohexanol were detected with better responses than could be expected from

their vapour pressures. This is probably due to the formation of hydrogen bonds between these molecules and the cyclodextrin matrix.
This last chapter also describes the synthesis of two polymerisable cyclodextrin derivatives. One of these derivatives was polymerised and the product was characterised by physical methods including GPC. Surprisingly, only hexameric fragments were observed together with aggregates of these hexamers, but no penta- and heptamers. The polymerisation reaction is probably self-terminating as a result of steric crowding in the stage of the hexamer. The latter oligomer showed extremely high binding affinities towards porphyrins ($K_b > 10^8\ M^{-1}$) which may be of interest for future applications of this compound in supramolecular sensing systems.

Samenvatting

Cyclodextrines zijn cyclische oligomeren van glucose die worden gevormd tijdens de enzymatische afbraak van zetmeel. De meest voorkomende natuurlijke cyclodextrines zijn α-, β- en γ-cyclodextrine die respectievelijk zijn opgebouwd uit 6, 7 en 8 glucose-eenheden. Deze moleculen hebben de vorm van een emmer zonder bodem. Op de randen van deze "emmer" bevinden zich hydroxylgroepen waardoor dit type verbindingen goed oplosbaar is in water. De holte van een cyclodextrine is apolair waardoor organische moleculen in waterig milieu kunnen worden gebonden.
In *hoofdstuk 1* zijn de eigenschappen van cyclodextrines uitgebreid beschreven samen met een aantal literatuurvoorbeelden waarin het gebruik van cyclodextrines in supramoleculaire systemen wordt toegelicht.
Hoofdstuk 2 behandelt de synthese van mono-gefunctionaliseerde cyclodextrines. In de gevolgde procedure werd de primaire (smalle) zijde van de cyclodextrines eerst beschermd met behulp van silylethergroepen. Deze methode had als voordeel dat de zo ontstane verbindingen goed oplosbaar waren in organische oplosmiddelen waardoor hun zuivering met behulp van flash-chromatografie mogelijk werd. Hierdoor konden de syntheses op grotere schaal worden uitgevoerd waarbij de producten (en tussenproducten) zuiver werden verkregen. Uitgaande van de gesilyleerde verbindingen werden vervolgens mono-gefunctionaliseerde cyclodextrines bereid die covalent konden worden verknoopt via een "spacer". Op deze manier werden twee cyclodextrine-homo-dimeren gemaakt. Door uit te gaan van twee cyclodextrinemoleculen van verschillende grootte (α-CD en β-CD) zijn ook twee cyclodextrine-hetero-dimeren gemaakt. Als de spacer die de twee cyclodextrines met elkaar verbond, lang was (acht methyleen-eenheden), bleek deze gecomplexeerd te worden in één van de twee holtes van het dimeer. Dit is met behulp van diverse NMR-experimenten aangetoond.

Eerder onderzoek in onze groep heeft aangetoond dat mangaan(III)porfyrines toegepast kunnen worden als katalysator in epoxidatiereacties van alkenen waarbij moleculaire zuurstof als oxidatiemiddel wordt gebruikt. Voor de activatie van de zuurstof moeten de porfyrines eerst gereduceerd worden door natriumformiaat. Dit laatste geschiedt door middel van rhodiumbipyridine-complexen als co-katalysatoren. In *hoofdstuk 3* is de synthese beschreven van twee cyclodextrinedimeren die een extra bindingsplaats bevatten in het spacergedeelte. Deze dimeren zijn interessant om te worden gebruikt in bovengenoemde katalytisch systeem, b.v. wanneer een porfyrine kan worden gebonden in de twee cyclodextrine-holtes en de derde bindingsplaats kan worden ingezet voor de binding van een substraat (alkeen) of de complexatie van de rhodium-co-katalysator. Als spacer is een mandvormig molecuul op basis van diphenylglycoluril gekozen, waarin dihydroxybenzeenderivaten gebonden kunnen worden. Voor de synthese van dit dimeer werd een cyclodextrine eerst mono-gefunctionaliseerd met behulp van α,α-dibromoxyleen zodat koppeling aan het mandvormige molecuul mogelijk werd. Het koppelingsproduct bleek zeer moeizaam te zuiveren te zijn. Omdat geen zuiver product verkregen werd, is dit molecuul niet verder op zijn katalytische werking onderzocht. Een tweede dimeer, waarin een bipyridinederivaat als spacer werd gebruikt, kon wel zuiver worden verkregen. Met behulp van deze spacer is een cyclopentadieenrhodiumverbinding tussen de twee cyclodextrines gecomplexeerd. Het zo ontstane rhodiumcomplex bleek in staat te zijn om porfyrines te reduceren. In aanwezigheid van een mangaanporfyrine en formiaationen kon met dit complex en moleculaire zuurstof nerol worden omgezet in nerolepoxide. Het aantal turnovers van het zo verkregen katalysator-systeem was echter te laag om interessant te zijn voor een industriële toepassing.

In *hoofdstuk 4* is in meer detail gekeken naar de binding van wateroplosbare porfyrines in cyclodextrinedimeren. Met behulp van ^{1}H-NMR-spectra in D_2O kon worden aangetoond dat dimeren die een flexibele alkylspacer bevatten, twee soorten complexen vormden. Een korte spacer resulteerde in een zogenaamd syn-complex waarin twee naast elkaar gelegen fenylgroepen van het porfyrine gebonden worden door de cyclodextrineholtes. Als een lange spacer werd gebruikt, bleek een mengsel aanwezig te zijn van het syn-complex en een complex waarin twee tegenover elkaar gelegen fenylgroepen van het porfyrine door de cyclodextrines zijn gecomplexeerd (anti-complex). In geval van een bipyridine als spacer werd een complex gevormd waarin twee porfyrines in een anti-oriëntatie gebonden zijn door twee cyclodextrinedimeren. De vorming van dit complex kon worden aangetoond met behulp van fluorescentiemetingen in combinatie met GPC-metingen en met behulp van NMR.

In hoofdstuk 4 is ook de binding van toluidinonaftaleensulfonaatderivaten in cyclodextrine homo- en hetero-dimeren beschreven. Deze niet-symmetrische gastmoleculen blijken door de cyclodextrine-hetero-dimeren zodanig gebonden te worden dat het naftaleengedeelte van de gast zich in het β-CD-deel van de gastheer bevindt en de kleinere tolueeneenheid in het α-CD-deel. Het binden van een substraat op een goed gedefinieerde manier zoals werd

waargenomen voor deze gastmoleculen, kan de basis vormen voor de ontwikkeling van een zeer efficiente regioselectieve katalysator

Hoofdstuk 5 beschrijft de synthese van drie cyclodextrinederivaten die een covalent-gebonden fluorescente (dansyl)groep bevatten Deze dansylgroep wordt gecomplexeerd door de cyclodextrineholte, maar kan door het toevoegen van een gastmolecuul (zoals adamantaancarbonzuur) worden verdrongen uit de holte De fluorescentie-intensiteit van de dansylgroep neemt hierdoor af en de mate waarin dit gebeurt, kan worden gebruikt om de hoeveelheid gastmoleculen in oplossing te bepalen Hierdoor is dit type cyclodextrine-moleculen te gebruiken als sensormoleculen De dansylgroep bleek echter zeer sterk gebonden te worden door de cyclodextrines waardoor het sensorsysteem niet erg gevoelig was Verlaging van de pH resulteerde in protonering van de dansylgroep waarna de verdringing door een gastmolecuul veel efficienter verliep Hierdoor konden zeer lage concentraties adamantaancarbonzuur in water worden bepaald Met behulp van NMR en UV-spectroscopie in combinatie met de bepaling van fluorescentielevensduren kon voor één van de drie verbindingen worden vastgesteld dat de dansylgroep zich niet in de holte maar buiten de holte bevond Dit molecuul gaf dan ook geen fluorescentieveranderingen te zien als adamantaancarbonzuur werd toegevoegd

In *hoofdstuk 6* is het gebruik van cyclodextrines in een gassensor beschreven Hierbij werd gebruik gemaakt van de quartzmicrobalansdetectietechniek Deze techniek berust op een trillend piezokwartskristal waarvan de frequentie afhankelijk is van de massa van het kristal Dit kristal werd bedekt met gesilyleerde cyclodextrines Als deze gastheermoleculen een gastmolecuul complexeren, zal de massa van het kristal toenemen waardoor een frequentie-verandering plaatsvindt Zo zijn, in theorie, zeer kleine massaveranderingen (nanogrammen) waarneembaar Er bleek een relatie te bestaan tussen de respons die werd waargenomen en de mate waarin een gastmolecuul past in een cyclodextrineholte De metingen lieten echter ook duidelijk zien dat een gedeelte van de gasten gebonden werd tussen de cyclodextrinemoleculen in plaats van in de holtes Opvallend was dat cyclohexanol en skatol, in zeer lage concentraties gedetecteerd konden worden Waarschijnlijk kunnen deze moleculen een waterstofbrug vormen met de cyclodextrinematrix waardoor een hogere respons verkregen werd, dan verwacht werd op grond van hun dampspanningen Tevens zijn in dit laatste hoofdstuk cyclodextrinederivaten beschreven die polymeriseerbare groepen bevatten Een van deze verbindingen werd gepolymeriseerd en na de afsplitsing van de beschermgroepen gekarakteriseerd met behulp van GPC De polymerisatie gaf als producten alleen cyclodextrine-hexameren en aggregaten hiervan, terwijl geen penta- of heptameren en hogere oligomeren aantoonbaar waren De polymerisatiereactie stopt waarschijnlijk zodra een hexameer is gevormd als gevolg van sterische hindering Het hexameer bleek een extreem hoge bindingsaffiniteit voor porfyrines ($K_b > 10^8$ M^{-1}) te bezitten hetgeen nieuwe toepassingen van dit type oligomeren in supramoleculaire sensorsystemen mogelijk maakt

Curriculum Vitae

Fokke Venema werd geboren op 26 februari 1966 in Harderwijk. Hij behaalde in 1983 het HAVO diploma en in 1984 het VWO diploma, beide aan het Christelijke College Nassau Veluwe te Harderwijk. Na het propaedeutisch jaar van de Amersfoortse Laboratorium School in 1985 te hebben afgerond, werd begonnen met de studie scheikunde aan de Rijks Universiteit Utrecht. Het propaedeutisch diploma werd behaald in augustus 1986. In december 1990 werd het doctoraal examen afgelegd, met als keuzevakken biochemie, NMR en biomoleculaire chemie (Leiden) en als hoofdvak fysisch-organische chemie (prof. dr. W. Drenth). In januari 1991 trad hij in dienst van de Katholieke Universiteit Nijmegen als assistent-in-opleiding. Het onderzoek dat onder leiding van prof. dr. R.J.M. Nolte en dr. M.C. Feiters tijdens deze aanstelling werd verricht, is beschreven in dit proefschrift. Sinds 1 september 1995 is de auteur werkzaam bij Organon Teknika te Boxtel.